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BEARING ON HEREDITY AND VARIATION

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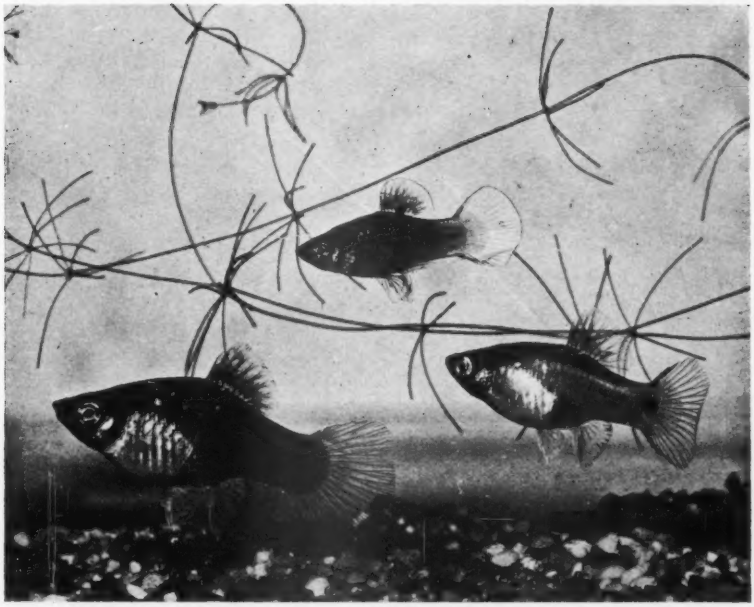

FIGURE 1
Volume 32, page 9
(M. GORDON, Genetics of *Platypoecilus maculatus*. IV.)

ON THE GENETICS OF SEVERAL TYPES OF SILVERING IN THE GUINEA PIG[1]

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Received October 19, 1946

INTRODUCTION

A SPRINKLING of white or light colored hairs among ones of a darker color in the coat of a mammal is variously known as silvering, roan or gray. At least four distinct patterns of this general sort are known in guinea pigs. The most familiar is one in which silvering, on the ground color determined by the rest of the genotype, is present at birth and does not change. A second type of stationary silvering is restricted to the blacks and browns and their modifications since it is due to the reduction of the yellow of the tortoiseshell pattern of intermingled yellow and dark pigmented hairs to white by certain genotypes. Third is a progressive type of silvering or grizzling, in which the color at birth is wholly that due to the rest of the genotype but scattered white hairs appear later and increase in number as the animal ages. Finally there is a retrogressive type which affects only intense browns. Scattered dark tipped pale brown hairs give a dingy appearance at birth which tends to be reduced or even to disappear in later pelages, expecially in females. The second type has long been understood. The others have been in need of further investigation because of paucity of published data or conflicting interpretations.

STATIONARY SILVERING

Description

The common silvering of the guinea pig varies enormously in degree and character. In its weakest development there are merely a few light or white hairs, usually on the mammary region of the belly. In higher grades, the belly may be more or less uniformly silvered or there may be irregularly distributed blotches of white within silvered areas. Extensive silvering of the belly is correlated with the occurrence of patches of silvering on the sides and middle of the back. More rarely slight silvering of the back occurs without silvering of the belly. In the highest grades, the belly becomes almost white, usually sprinkled here and there with dark hairs or blotches, and the back becomes strongly silvered. The head and feet show little or no silvering even in the highest grade, as in roan horses. Red hairs are more conspicuously diluted in silvered areas than are black ones. It is sometimes difficult to decide whether a tortoiseshell with apparently dilute yellow hairs, intermingled with intense black, but no white, is to be considered a low grade silver or not. Silvered self reds and yellows are also difficult to classify unless the silvering is strong.

[1] This investigation was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Previous Accounts

CASTLE (1912) produced a strongly silvered strain from low grade silvers by selection but arrived at no factorial interpretation. WRIGHT (1917) stated (on the basis of a few crosses between a strongly silvered inbred strain, No. 34, and nonsilvers) that F_1 usually showed slight silvering on the belly but that there was no clear cut segregation in F_2. IBSEN (1932) tentatively attributed what seems to be the same pattern to an incompletely dominant gene, *Ro* (roan). He gave no data, however. PICTET and FERRERO (1940) have published extensive data on silvering which seems from the description and photographs to be identical with that considered here. They ascribe whitening of the belly (blanchiment du ventre, partiel ou total) to a dominant gene, *V*. Silvering of the back first appeared in their colony on a whitebellied animal. They found it to be separable, however, and describe it as behaving as a simple recessive (*d*) in crosses with nonsilvers, and capable of increase by selection. The effect of *d* is referred to in most places as restricted to the back but in one place (p. 83) it is stated that it may reach the ventral surface. The evidence for their two factor interpretation will be discussed later.

Experiments

The silvering of the following experiments traced, except where otherwise stated, to an inbred strain, No. 13, in which the average extent of colored areas was so small (about 2 percent of the coat, WRIGHT and CHASE 1936) and so nearly restricted to the head that silvering was rarely evident. Silvering of all grades appeared, however, in the descendants of crosses with a tortoiseshell strain. No analysis of the early segregating generations is practicable; partly because the young were not graded with sufficient care in this respect and partly because the large amount of white in many of them made classification highly uncertain. It is, however, important to give an idea of the range of variability among those descended wholly from animals with more than slight silvering and from crosses between these and inbred strain 13. In table 1, a distinction is made between matings (A) in which both parents had more than slight silvering and (B) ones in which one or both parents had only slight, if any, silvering, though descended wholly from the former group. Class C consists of the crosses between either of these (strain I-13) and strain 13. Among the offspring a distinction is made between those with 50 percent or more of color in the coat, including color on the posterior belly, and the rest in which classification is of little value. The former are classified on the basis of descriptive terms used in the records, such as "no silver," "very slight," "medium," "strong," and "very strong." In most cases, however, "medium" and "strong" here mean merely that silvering was ascribed to the belly alone or to both belly and back, respectively. It is probable from later results that more careful examination would have raised most, if not all, of those called "nonsilver" to the category "very slight silver."

The most important conclusion from this table is that animals tracing exclusively to conspicuously silvered ancestors may range from ones with at

least no easily recognized silvering to ones with very strong silvering.

A series of experiments to explore the mode of inheritance was begun by mating medium, strong, or very strong silvers from strain I-13 with nonsilvers

TABLE 1

Distribution of grades of silver in strain I–13, from parents (A) both of which were at least of medium grade, or (B) of which one or both were no more than slightly silvered but were derived from the preceding or (C) one parent was derived from (A) or (B), the other from strain (13), the original source of silvering in I-13.

PARENTS	NO MATINGS	MORE THAN 50% COLOR IN COAT							LESS THAN 50% COLOR		
		% OF YOUNG OF EACH GRADE						NO. YOUNG	% NO SILVER	% SILVER	NO. YOUNG
		NO SILVER	V.SL.	SL.	MED.	STR.	V. STR.				
A I-13×I-13	30	3.1	1.8	6.6	36.1	41.0	11.4	227	63.0	37.0	46
B I-13×I-13	19	16.1	1.6	14.5	32.3	29.0	6.5	62	78.0	22.0	41
C I-13×13	15	10.7	4.8	11.9	40.5	30.9	1.2	84	70.4	29.6	54
Total	69	7.0	2.4	9.1	36.5	36.7	8.3	373	70.2	29.8	141

from various sources. Grades of silver have been assigned at birth to back and belly separately to test the hypothesis of PICTET and FERRERO that silver in these areas is determined by independent genes.

Grade 0	no silver
Grade 1	trace of silvering (irregular dilution or few scattered white hairs)
Grade 2	slight but unmistakable silvering in a small area
Grade 3	silvering over less than half the area
Grade 4	strong silvering and blotching with white over more than half the area
Grade 5	white predominates

Doubtful grades were checked at weaning if possible. In some cases a trace of silvering was apparent at weaning which had not been observed at birth and in other cases an apparent trace at birth could not be detected later. Changes were rarely made in the higher grades.

The principal results are given in table 2. The grades of back and belly are here averaged: averages 0.5 and 1.0 are called 1, 1.5 and 2.0 are called 2, etc.

The results in F_1 indicate that silver is more nearly recessive than dominant. In five matings, no trace of silver was detected in 69 percent of the 39 young and only one individual was assigned grade two. Another F_1 mating gave high grade silvers as well as nonsilvers and low grades. The nonsilver parent in this case, however, may well have been heterozygous since silvering has been rather widely distributed in the colony.

Crossbreds showing no silver or only a trace have been backcrossed generation after generation to animals of I-13 that were strongly silvered at least on

the belly. If only one major factor is involved, 50 percent of the F_1 type should appear in each generation in spite of the continued attempt at dilution. With two major factors only a little more than 25 percent should continue to be of grades 0 and 1, while if multiple minor factors are involved backcrossing should soon produce a strain like I-13 in spite of selection of one parent for low grade. The first of these possibilities is the one that was realized. There were

TABLE 2

Distribution of grades of silvering from matings derived from outcross of silvers of I-13 to miscellaneous nonsilvers.

	PARENT (LOWER)		PARENT (HIGHER)		NO. MATINGS	GRADES OF SILVERING						TOTAL	% 0–1	% 2–5
						0	1	2	3	4	5			
F_1	*SiSi* (Misc)	0	*sisi* (I-13)	4.0	5	27	11	1				39	97.4	2.6
F_1	*Sisi* (Misc)	0	*sisi* (I-13)	3.5	1	2	1	2	2	1		8	37.5	62.5
1BC	*Sisi* (F_1)	0.1	*sisi* (I-13)	3.7	10	37	25	17	18	7	5	109	56.9	43.1
2BC	*Sisi* (1BC)	0.5	*sisi* (I-13)	3.9	10	14	14	13	12	7	1	61	45.9	54.1
3BC	*Sisi* (2BC)	0.5	*sisi* (I-13)	2.9	3	9	8	3	3	4	2	29	58.6	41.4
4BC	*Sisi* (3BC)	0.0	*sisi* (I-13)	3.5	1	2	6		3	3	1	15	53.3	46.7
5BC	*Sisi* (4BC)	0.5	*sisi* (I-13)	4.5	1		1	1		1	1	4	25.0	75.0
Total BC	*Sisi*	0.3	*sisi*	3.9	25	62	54	34	36	22	10	218	53.2	46.8
$(F_1)^2$	*Sisi* (F_1)	0.0	*Sisi* (F_1)	0.0	2	9	3	2				14	85.7	14.3
$(1BC)^2$	*Sisi* (1BC)	0.5	*Sisi* (1BC)	0.5	5	10	28	4	5	2		49	77.6	22.4
$(2BC)^2$	*Sisi* (2BC)	0.0	*Sisi* (2BC)	1.0	1	1	1	1		1		4	50.0	50.0
$(3BC)^2$	*Sisi* (3BC)	0.4	*Sisi* (3BC)	0.9	2	9	7	2				18	88.9	11.1
Total $(BC)^2$	*Sisi*	0.4	*Sisi*	0.5	10	29	39	9	5	3		85	80.0	20.0
$(BC)^2$	*sisi* (BC)	2.1	*sisi* (BC)	3.1	3		1	2	7	3	6	18	5.6	94.4
2 BC	*sisi* (BC)	3.0	*sisi* (I-13)	4.3	5		3	5	6	4	2	20	15.0	85.0
Total	*sisi*	2.6	*sisi*	3.7	8		4	7	13	6	8	38	10.5	89.5
	Si- $(BC)^2$	0.6	*Sisi* (BC)	0.6	2	10	7					17	100.0	0.0
	Sisi $(BC)^2$	0.1	*Sisi* (BC)	0.1	2	8	13	4	4	3		32	65.6	34.4
$(BC)^2$	*Sisi* (BC)	0.6	*sisi* (BC)	2.1	4	7	3	2	4	5		21	47.6	52.4

56.9 percent of grades 0 and 1 among 109 young from the first backcross and 49.5 percent of these grades among the same number of young from later backcross generations.

The hypothesis that silvering depends on an incomplete recessive (to be called *si*) has been confirmed by other tests. Backcross silvers of the higher grades (2–5) mated with I-13 or with each other have given no nonsilvers and no more of grade 1 than can be accounted for from the distribution in I-13 itself, and are thus indicated to be *sisi*. Matings between nonsilvered parents or ones with only a trace, whether from F_1 or the first to third backcross gave largely (80 percent) nonsilver or grade 1, but 20 percent of grades 2 to 4, indicating that these matings were of type *Sisi*×*Sisi*.

A few matings which do not fall into the preceding categories gave results easily interpreted as in harmony with the conclusion that there is only one major pair of alleles.

TABLE 3

Distribution of backcross progeny by sex and type of mating with respect to sex.

♀(♀♂)	MATINGS	OFFSPRING				TOTAL
		FEMALES		MALES		
		0–1	2–5	0–1	2–5	
R(RD)	6	16	15	15	18	64
R(DR)	4	9	7	12	11	39
(RD)R	2	5	2	3	3	13
(DR)R	13	24	16	32	30	102
Total	25	54	40	62	62	218

The progeny of the backcross matings are classified in table 3 by sex of parents and of offspring. R(RD) means a mating of recessive (silver) female by a male from recessive mother and dominant father. The other possibilities, R(DR), (RD)R and (DR)R are to be interpreted similarly. Matings of type R(RD) and R(DR) test the possibility of partial sex-linkage on the preferred hypothesis that males are heterogametic while (RD)R and (DR)R are tests on the less probable hypothesis that females are heterogametic. The absence of significant differences in the distribution of grade classes (0–1) and (2–5) among male and female progenies indicate that there is no sex-linkage of any sort.

It is evident that the range of silvering in heterozygotes overlaps both homozygotes. To get a more accurate idea of the amount of overlap than can be obtained merely by inspection of table 2, the 459 individuals in this table have been apportioned to genotypes and grades in as nearly exact Mendelian ratios as possible from each type of mating, and with a minimum of overlap. Table 4 shows the results.

TABLE 4

Apportionment of individuals of table 2 to genotypes on the assumption of as nearly exact Mendelian ratios as possible and a minimum of overlap.

PARENTS	NO. MATINGS	PROGENY									TOTAL
		SiSi	*Sisi*			*sisi*					
		0	0	1	2	1	2	3	4	5	
SiSi×*sisi*	5		27	11	1						39
Sisi×*sisi*	30		71	52		6	38	42	28	10	247
Sisi×*Sisi*	12	29	8	51		1	13	9	6		117
sisi×*sisi*	8					4	7	13	6	8	38
SiSi×*Sisi*	2	9	2	7							18
Total	57	38	108	121	1	11	58	64	40	18	459
% within genotypes		100.0	47.0	52.6	0.4	5.8	30.4	33.5	20.9	9.4	
Mean		0.0		0.5				3.0			

There is no clear evidence that *SiSi* ever showed any silvering although in one mating, parents both called grade 1, *SiSi* or *Sisi*, produced ten young all of grades 0 or 1.

The heterozygotes must be supposed to be distributed in varying proportions between grades 0 and 1 and only very rarely grade 2. In the total, 47 percent of *Sisi* had no detectable silvering and 53 percent had a trace. Genotype *sisi* is indicated to cover the range from a trace to very strong silvering with a mean at 3.0, medium silvering.

Table 5 shows the relation between the grades of silvering of back and belly among the 218 young from the backcrosses.

TABLE 5

Joint distribution of grades of silvering of back and belly in the backcross population.

		GRADE OF BELLY						TOTAL		IN TOTAL	IN UPPER 50%
		0	1	2	3	4	5				
	0	62	28	22	12	1		125	Back	1.03±.09	2.02±.13
	1	2	2	3	13	2		22	Belly	1.96±.11	3.38±.08
Grade	2			5	16	9		30	Correlation	.80±.024	0.68±.054
of	3			1	8	14	3	26			
Back	4					5	6	11			
	5						4	4			
	Total	64	30	31	49	31	13	218			

The strong correlation, .80±.024, between back and belly, demonstrates again that there can not be random assortment of two major genes, acting separately in these two regions. The rather large number (63) with silvering of the belly but not of the back as compared with the small number (2) in which the opposite was true, seems merely to reflect an average difference in threshold. This difference might to some extent account for the dominance of ventral silvering and recessiveness of dorsal silvering, noted by PICTET and FERRERO, but only as different effects of the same gene, not of independent genes.

The correlation between back and belly in silvers, homozygous in *si* but segregating in minor factors, may be estimated roughly by considering only the upper 50 percent of the backcross population (those in which the sum of dorsal and ventral grades is 3 or more, plus seven from the class graded 2 on the belly, 0 on the back). The correlation in this group is .68±.054, which indicates that 68 percent of the variability in silvers of genotype *sisi*, but otherwise heterogeneous, is due to factors (nongenetic or minor genetic), that act alike on back and belly, leaving only 32 percent to factors that act more locally. That there is considerable nongenetic variability is indicated by the frequency of marked asymmetry and other irregularities on both back and belly and is, of course, to be expected of a character, the very nature of which implies different thresholds among hair follicles that all presumably have the

same genetic constitution. The efficacy of selection indicates, however, that minor genetic factors play some role.

Varying degrees of silvering have appeared in a stock of reds and yellows. The character is manifested in these by a usually rather slight but apparently rather uniform dilution of the color of the back, associated with a sprinkling of white hairs which is less conspicuous than on a black ground, and with a marked reduction of color on the belly which appears light yellow, blotched yellow and white, or solid white, depending on the grade. This silvering was independent of strain 13 in origin. The grading within this stock has not been sufficiently accurate for presentation but strong silvering has seemed to behave on the whole as recessive. Crosses of strongly silvered reds with silvers of strains I-13 or 13 have given silvered black-red tortoise shells of the following grades:

	MATINGS	0	1	2	3	4	5	TOTAL
White bellied light red×silver (I-13, 13)	5	0	2	3	2	2	9	18

From this result, there seems little doubt that the same recessive gene *si* is involved.

Not all unexpectedly dilute reds and yellows are *sisi*, however. Two unexpectedly pale creams (*ee* $c^k c^r$ *FF* and *ee* $c^k c^a$ *FF*), from an experiment in which the amounts of yellow pigment in diverse genotypes were being determined, were mated with silver from I-13. These pale creams did not have bellies appreciably lighter than their backs. The results (included in F_1 in table 2) indicate that these animals were *SiSi*.

	MATINGS	0	1	2	3	4	5	TOTAL
Pale cream×silver (I-13)	2	7	2	1				10

Discussion of previous interpretations

The results obtained in these experiments agree with the earlier statements by the author (1917), IBSEN (1932) and PICTET and FERRERO (1940) that silver may have at least some degree of dominance, manifested by silvering of the belly in the crossbreds. The gene, called *si* here, may be the same as that listed by IBSEN with the symbol *Ro*. It is obvious, however, that the gene is quite unusable as a dominant in the experiments described here, not only because about half of the heterozygotes show no silvering whatever, but those that are called silver have such a slight trace that their classification is often doubtful. The gene can be used as a recessive on the other hand with about the same degree of reliability as in the case of the spotting factor, *s*, by drawing an arbitrary line according to the expected ratio (cf. WRIGHT 1941).

The relation of *si* to PICTET and FERRERO's *V* and *d*, if any, is a puzzling matter. It seems unlikely that two apparently identical series of variations should have nothing in common genetically yet it is certain that the present results can not be explained by separate genes for silvering of back and belly and on the other hand PICTET and FERRERO's published results appear to

substantiate their interpretation and certainly do not lend themselves readily to interpretation in terms of *Si,si*. There are, however, certain difficulties in the interpretation. The original dorsal silver (which also had ventral white and was considered to be *VV dd*) was mated with a ventral white which had produced normals and was therefore assigned the formula *VvDD*. The progeny, 16 in number, were all ventral whites, presumably *VvDd* and *VVDd*. Two matings among these gave the following results. In the authors' terminology *Vbl* means ventral white alone, *Vbld* is ventral white associated with dorsal silvering and *arg* is dorsal silvering alone.

MATING	*Vbl*	*Vbld*	NORMAL	*arg*	TOTAL
Vbl(F_1)×*Vbl*(F_1) *VvDd* ×*VvDd*	17 (9/16)*V-D-*	10 (3/16)*V-dd*	0 (3/16)*vv D-*	6 (1/16)*vvdd*	33

The most striking thing here is the absence of normals (*vvD-*) although the ratio as a whole is far from good. It should be said, however, that these matings gave much the poorest fit of any reported in the paper. They contribute about one-third of the total χ^2 referred to later. Six later matings are reported as between ventral whites without dorsal silvering, but with dorsally silvered young (necessarily *VvDd*×*VvDd*).

	Vbl	*Vbld*	NORMAL	*arg*	*Total*
Vbl(*VvDd*)×*Vbl*(*VvDd*)	105	36	0	12	153

Here again there are no normals, *vvD-*, although this class should constitute 3/16 of the young from this type of mating. The numbers in the other classes are however in excellent agreement with expectation. The authors advance the hypothesis that the normal genotype is lethal when produced by *VvDd* ×*VvDd*. This is supported by the smaller size of litter in this case (2.25) as compared with 2.785 from 89 litters from *vvDd*×*vvDd* (which produces the same kinds of normals). The difference almost exactly accounts for the missing normal young.

According to this hypothesis, the union of a *vd* sperm with a *vD* egg, or of a *vD* sperm with either a *vd* or a *vD* egg gives lethal results but only if both parents are *VvDd*. Compare for example the results of the following matings (from which parents of the anomalous lethals were at least in part derived) which show that *VvDd* individuals of either sex produce the four expected types of germ cells in the expected 1:1:1:1 ratio and that two at least of the three alleged types of lethal unions give viable young when only one parent is *VvDd*.

MALE	FEMALE	*Vbl*	*Vbld*	NORMAL	*arg*	TOTAL
Vbl(*VvDd*)	×*arg*(*vvdd*)	17	18	18	14	67
arg(*vvdd*)	×*Vbl*(*VvDd*)	8	5	6	8	27
Total		25	23	24	22	94

The burden of proof on such an hypothesis seems so great that it would seem preferable to interpret the matings between *Vbl* parents cited above, as

ones in which both were homozygous in a gene for silvering (*sisi*) which might manifest its action in any of the three forms *Vbl*, *Vbld* or *arg* according to minor factors, genetic or otherwise.

Here, however, we must take cognizance of the closeness with which the observed results are matched by the calculated ones. The data are collected in tables 16 and 17 in PICTET and FERRERO'S paper. A series of 48 matings, beginning with ones between extracted dorsal silvers (*vvdd*) and normals (*vvD-*) are reported in table 16 as giving only these two classes, in close agreement with expectation for every individual mating. Similarly close agreement is exhibited by the 47 matings (table 17) in which one or both parents had gene *V*. An analysis of the segregating progenies from these matings gives the following results:

	DEGREES OF FREEDOM	χ^2	PROBABILITY
Table 16	27	3.5	.999,996
Table 17	62	17.7	.999,999,8
Total	89	21.2	.999,999,999,99

According to the descriptions, both whitening of the belly and dorsal silvering varied from mere traces to strong; and dorsal silvering (in the category *arg*) might invade the belly. It would seem that there must have been intergrading of all four categories and many doubtful cases. One can only conclude from the extraordinary goodness of fit that doubtful cases were resolved in favor of the expected ratios.

It is possible to account for PICTET and FERRERO'S results in terms of *Si* and *si* but this means little since normals may be either *SiSi* or *Sisi*, *Vbl* and *arg* either *Sisi* or *sisi* and even *Vbld* may occasionally be *Sisi* as well as *sisi*. A record of degree of silvering, rather than mere occurrence is necessary for an analysis. The possibility that the heterozygotes overlap both homozygotes can hardly be tested adequately without determining the resistance to dilution of the F_1 type by repeated backcrossing to pure recessives, supplemented by breeding tests of supposed recessive segregants. It may be concluded that the postulated independent genes for ventral (*V*) and dorsal (*d*) silvering cannot be accepted as demonstrated and that it is at least possible that the same incomplete recessive (*si*) may underlie all stationary silvering of the type which may be manifested on all ground colors.

SYNTHETIC STATIONARY SILVERING

In genotypes $SSe^{p}e^{p}$ or $SSe^{p}e$ there is a sprinkling of yellow hairs on a ground of one of the darker colors in the tortoiseshell pattern (IBSEN 1919), with more yellow on the average in $SSe^{p}e$ than in $SSe^{p}e^{p}$ (CHASE 1939). To some extent in $Sse^{p}e^{p}$ or $Sse^{p}e$ and to a greater extent in $sse^{p}e^{p}$ and $sse^{p}e$, yellow tends to occur in large areas resembling in form and location the white areas which also appear in these tricolors (WRIGHT (1917), ILJIN (1928), CHASE (1939)). There is, however, considerable sprinkling of yellow in the black areas and of black in the yellow areas. It has long been known that

replacement of gene C by lower alleles tends to reduce the intensity of yellow disproportionately and in certain cases (c^rc^r, c^rc^a, c^kc^rff, c^kc^aff, c^dc^rff, c^dc^aff) the reduction is to white (WRIGHT 1915, 1927). There may be considerable resemblance to the preceding type of stationary silvering as manifested in silvered blacks and browns, but not of course in silvered reds and yellows.

PROGRESSIVE SILVERING (OR GRIZZLING)

Lambert's results

A type of silvering of the guinea pig that first appears after the first pelage and which progresses with age, like gray hair in man, and gray (as opposed to roan) in horses, seems to have been described only by LAMBERT (1935). It has been observed in my colony only in a few animals, kindly presented by DR. LAMBERT, and in descendants of these.

The character consists of the occurrence of totally white hairs, scattered principally over the posterior region of the back but in extreme cases over all the back and sides and to a lesser extent on the head and belly. At birth the animals may be of any color, depending on the residual genotype. According to LAMBERT, 40 days is the earliest at which white hairs appear (in the posterior dorsal region). LAMBERT's principal results (substituting grizzled for his term silver) were as follows:

MATING	GRADE OF GRIZZLING					TOTAL
	NONE	TRACE	LOW	MEDIUM	HIGH	
Grizzled×grizzled		1	6	10	22	39
Grizzled×nongrizzled (outcross)	12	2				14
Grizzled×heterozygote	35	28	24	21	13	121
Heterozygote×heterozygote	17	7		5	1	30

These results point clearly toward an incompletely recessive major factor (LAMBERT's interpretation) although reports of breeding tests of the supposed segregants are needed to clinch the demonstration. Thus in a cross between inbred normal and polydactyl strains of guinea pigs an apparent one factor difference, from evidence more extensive than that above, was completely disproved on testing the supposed segregants (WRIGHT 1934). Accepting provisionally the one factor interpretation, it may be noted that the amount of overlap of the heterozygotes with both homozygotes is remarkably similar to that inferred earlier in this paper for stationary silvering. In both cases the situation is also very similar to that found for spotting (*s*) (WRIGHT and CHASE 1936). LAMBERT gives no symbol. I shall use *gr* (grizzling) to avoid confusion with stationary silvering (*si*) and to suggest its progressive character.

Experiments

My own data are not very extensive but confirm LAMBERT's interpretation as far as they go. The pure stock derived from his stock produced 54

young which were saved beyond weaning. All of these became grizzled, some as early as two months. A few were ungrizzled at four months but became grizzled, usually of a high grade, later.

Reciprocal crosses to ungrizzled animals of my colony gave 21 young which were saved to six months or more. Only one of these developed a trace of grizzling (first noted at nine months). These include a mating to a silver of stock I-13. At birth one of the young showed slight silvering of the belly, three showed traces on the belly, five showed no silvering and 13 others were born before careful grading began, but no silvering was recorded and presumably no more than a trace was present if any. Four of the young, including one with a trace of ventral silvering, were kept to ten or more months. None developed any dorsal grizzling. Thus there appears to be no relation between stationary silvering and progressive grizzling.

A backcross to the pure grizzled stock gave 12 nongrizzled (at six months or more) to eight grizzled (two slight, six stronger). Two of the nongrizzled were backcrossed again to pure grizzled and gave five nongrizzled (at six months or more) to two slightly grizzled. These results tend to indicate a normal gene that behaves as a unit in withstanding successive attempts at dilution by backcrossing to the pure recessive strain. Analysis of these progenies by sex of parents and offspring gives no indications of sex-linkage. Using $R(DR)$ for recessive (grizzled) female by male from dominant female and recessive male and $(DR)R$ for the reciprocal backcross, the results were as follows.

TABLE 6

Backcross progeny classified by character, sex and nature of the mating.

MATING	FEMALE		MALE		TOTAL	
	NON-GRIZZLED	GRIZZLED	NON-GRIZZLED	GRIZZLED	NON-GRIZZLED	GRIZZLED
R(DR)	3	4	4	2	7	6
(DR)R	6	3	4	1	10	4
Total	9	7	8	3	17	10

Matings between heterozygotes gave eight nongrizzled (at six months or more) to three grizzled. Matings between heterozygotes and pure nongrizzled gave 24 young all nongrizzled. Extracted grizzled mated *inter se* have given 14 that were grizzled at six months or later and three not grizzled when last examined (six months in two cases, nine months in the other). As three of those ultimately recorded as grizzled showed no grizzling at six, nine and ten months respectively, the nongrizzled might have become grizzled if they had lived long enough. It is obvious that the grizzling extracted from the outcrosses was not as strong as that from LAMBERT'S selected stock.

The increase in grizzling with age is shown in table 7 for the pure stock, for the segregants which ultimately become grizzled and for the descendants of extracted grizzled. Grade 0 is nongrizzled, 1 means a trace, and 2 slight, 3

medium, 4 strong and 5 very strong grizzling. The nongrizzled are included in the averages.

In conclusion, there seems little doubt that there is one major pair of alleles (*Gr*, *gr*) although more extensive tests of segregants would be desirable. The degree of effect is undoubtedly subject to minor factors. With the usual modi-

TABLE 7

Increase in grizzling with age. The number of animals examined, the number nongrizzled and the average grade are shown by two month periods to 17 months, for ages 18 to 23 months and for 24 months and over.

		AGE IN MONTHS										
		0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	24–
LAMBERT Stock	number		14	27	27	17	10	8	13	7	12	3
	nongrizzled		4	1	0	0	0	0	0	0	0	0
	av.	0	0.9	1.5	2.4	2.8	2.7	2.9	3.2	3.6	3.5	4.3
Segregants	number			7	7	3	7	3	2	3	4	1
	nongrizzled			3	2	1	0	0	0	0	0	0
	av.	0	0	0.7	1.3	1.3	2.6	2.3	2.0	3.0	2.3	2.0
Extracted Strain	number			20	10	10	5	3	3	0	2	0
	nongrizzled			12	3	2	1	0	0		0	
	av.	0	0	0.4	1.0	1.1	1.4	1.7	2.0		1.5	

fiers, grizzling is completely or almost completely recessive and even in homozygotes may not appear until the animal is nearly a year old. A residual heredity can be built up by selection that enables grizzling to appear at two months or even less and to become very strong in time and which enables a trace of the character to appear in heterozygotes.

RETROGRESSIVE SILVERING OR DINGINESS OF BROWN

IBSEN (1932) included in his list of variations of the guinea pig a white tipping for which he suggested an incompletely dominant factor (*W*). "*W* causes the distal half of all chocolate (*b*) hairs, on the back especially, to become white, provided the animal carries *C*, the factor for full color (red is present). It has no effect in c^r (absence of red) animals. The effect on black hair (*B*) is very slight; homozygotes have a few white tipped hairs on the head, while heterozygotes do not show any white tipping. Red hair (*CCee*) is entirely unaffected by *W*. There is also a sex-limited effect in that nearly all females gradually lose their white tipping while males tend to retain it." No data were given.

A variation with some of the above characteristics has been present in browns (chocolates) of my colony and has been referred to briefly in a previous paper (WRIGHT 1942). EATON (1943) has described a similar condition as occurring in browns of the stock of the U. S. BUREAU OF ANIMAL INDUSTRY

(from which my stock split off in 1926) and as following an irregular mode of inheritance. There are a number of respects in which the condition in my stock differs from IBSEN's account while agreeing more nearly with EATON's description. Not all brown hairs are affected, even on the head, except in the more extreme cases. There is instead a sprinkling of light colored hairs among ones of full intensity. Moreover brown seems never to be reduced to white in any part of the hair in my stock, even in the highest grades, nor is the lightest portion of the hairs at the tip. Some two or three mm. at the tip of the hair is of full or nearly full intensity. Below this, some hairs merely show a band in which brown is reduced to a color comparable to that of a very pale brown (*E bbc^{d}c^{d}ppFF*) followed by less dilute brown to the base. Other hairs are uniformly pale brown except for the tip. In the most extreme grades all hairs are pale brown with dark tips. According to EATON dilution is more extreme in brown agoutis than in browns and this dilution occurs only below the yellow subterminal band of the agouti pattern. The term white tipping obviously does not apply to the condition in these stocks. The term dinginess gives the general impression given by these browns in contrast with intense browns and will be used here for this type of variation.

It was noted in the previous paper that this condition could not be transferred to browns with $c^k c^k$ (c^k is a higher allele of albinism than c^r referred to by IBSEN) or with *pp*, extreme diluter of brown and sepia but could be transferred to browns with *ff* (i.e. *E-bbCCPPff*). Replacement of *F* by *f* has no appreciable effect on browns or sepias in the presence of *P* although it removes these colors in combination with *pp*. The paradoxical effect of dinginess in reducing brown only when it would otherwise be most intense made further study of its effects of interest.

Experiments

Strong dinginess of brown first appeared in some segregants from a cross made by DR. E. C. COLIN between the same black-red-white tricolor strain No. 13 that was the source of stationary silvering and a stock (C) which carried brown. DR. COLIN began experiments with these in 1930. He developed a strongly dingy strain and mated one of these with a pink eyed dilute brown tricolor (*sse^{p}e^{p}aabbc^{k}c^{k}FFpp*) from a strain (B) that was the most inbred strain of browns in the colony at the time. F_1 consisted of intense browns with no dinginess. Absence of dinginess persisted through three successive back crosses to the dingy stock in more than 50 percent of the young (total 61 non-dingy, 19 slightly dingy and 30 strongly dingy). Extracted dingies, mated *inter se* gave four nondingy, 18 slightly dingy and 94 strongly dingy. These results indicate that nondingy differs from dingy primarily in one major dominant gene. From later results, however, it appears probable now that this "dominant" gene was the well known recessive *p*, introduced in the original mating.

A stock (*S*) of browns from DR. COLIN's experiments was carried on for a number of years before an attempt at semiquantitative analysis was made by

means of grades. Grade 0 is complete absence of dinginess. In grade 1, there is a trace of dinginess, restricted to scattered hairs on the cheeks and perhaps the nape. In grade 2, there is unmistakable dinginess of scattered hairs, restricted to head and nape. In grade 3, scattered dingy hairs are found on the back but substantial nondingy areas are also present. In grade 4, all parts of the back (as well as the head) are sprinkled with dingy hairs and there may be some dinginess on the belly. Grade 5 was adopted later for cases in which practically all hairs are affected.

Many animals of this stock, especially in the earlier generations, had only small areas of color on a white ground due to *ss*, derived as in the case of the silver stock, from strain 13. Grading of these presented less uncertainty than in the case of silver since dinginess can be graded from observation on the head, the commonest location of colored spots in extreme piebalds in contrast with silver, which cannot be graded satisfactorily in the absence of color on the posterior belly. There is no indication that the dinginess of colored spots is at all affected by genes *S*, *s*. Nearly all animals have been graded at birth. A second grade has usually been given when possible at about 30 days, when low grade dinginess is sometimes more conspicuous than at birth. Following are the averages of live born and still born intense browns at birth and of those that reached weaning, from matings that trace wholly to animals of grades 3 and 4 in the stock S referred to above and similar averages from the progeny of animals of grades 3 to 5 that segregated from outcrosses (SP, SK, SA) to other stocks referred to later. In both cases, matings that produced dilutes (c^kc^k etc.) are excluded. All were probably *bbC-PPFF*, but undoubtedly some individuals were heterozygous for albino alleles (Cc^k, Cc^a etc.). (Table 8.)

Descendants of high grade dingies

TABLE 8

Mean grades of dinginess at birth among live born and still born young, and at weaning, from the high grade stock S and from matings between high grades deriving from outcrosses.

TIME OF GRADING	STOCK S		EXPERIMENTS SP, SK, SA	
	NO.	MEAN SE	NO.	MEAN SE
Birth (live born)	326	2.93±.06	182	3.02±.09
Birth (still born)	84	2.36±.12	64	2.61±.13
30 days	233	3.00±.06	139	3.32±.09

The grades of still born young are significantly lower than those of the live born young, possibly because of difficulties in grading in some cases (short hair, wetness, etc.). It has seemed best to exclude them from the data. The correlation between grade at birth and that at weaning was .75±.03 in series S and .85±.02 in the later group. The difference between the grades was not significant in the former but is in the latter.

The grades at weaning are undoubtedly more reliable than those at birth

but it has not seemed desirable for the purposes of this paper to exclude the grades of live born young when no later grade was given. The grade in all later tables is thus the last grade up to weaning of the live born young.

There was segregation of E and e^p in these experiments. The average grades of young without red spotting (largely E) and with red spotting (e^pe^p) are as follows in the two groups.

	STOCK S		EXPERIMENTS SP, SK, SA	
	NO.	MEAN SE	NO.	MEAN SE
Brown	111	2.96±.10	100	3.36±.12
Brown-red	215	3.02±.07	87	3.09±.11

It is obvious that there are no consistent differences and no distinction will be made later.

The full distribution of grades in these two groups is given below by sex.

TABLE 9

Distribution of grades by sex in stock S and among offspring of high grades derived from outcrosses.

		PERCENTAGE						NO.	MEAN SE
		0	1	2	3	4	5		
Stock S	♂	1.2	4.1	18.7	35.1	40.9	—	171	3.11±.07
	♀	2.6	7.1	24.5	31.0	34.8	—	155	2.88±.08
	Total	1.9	5.5	21.5	33.1	38.0	—	326	3.00±.06
SP, SK, SA (parents 3–5)	♂	1.1	6.5	14.1	25.0	44.6	3.7	92	3.32±.11
	♀	0	11.6	13.7	28.4	40.0	6.3	95	3.16±.11
	Total	0.5	9.1	13.9	26.7	42.3	7.5	187	3.24±.08

The males are slightly higher in grade in both stocks but the difference is of somewhat doubtful significance. If both stocks are combined the difference (.191±.092) is 2.1 times its standard error.

The wide variability of stock S suggests great genetic heterogeneity. The parents in the 58 matings in which both were graded (308 young) ranged from grade 1 to 4. The correlation between father and offspring was +.280, between mother and offspring +.086, giving an average of +.183. There was a low correlation between the parents, +.212, due to a considerable number of brother-sister matings rather than to intentional assortative mating. On the simplest hypothesis (no dominance, no interaction effects, a constant tendency to inbreeding) the correlation between parent and offspring is $(1/2)h^2(1+m) = .183$ where h^2 is degree of determination by heredity and m is the correlation between the genotypes of parents. This indicates less than 37 percent determination by heredity. The correlation between parents (often littermates) may include a nongenetic component. The nongenetic variance includes that

due to inconsistencies of grading, but the scale is such a coarse one that this can hardly be the major component.

Mated animals have been graded at the time of birth of each litter. Table 10 shows the averages for males and females of each initial grade. The males are distinctly less dingy at four or five months than at first but rise slightly in

TABLE 10

Grades of dinginess at successive ages of males and females of each initial grade.

		AGE IN MONTHS											
		0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	24–	6–40
	no.		1	49	37	24	24	19	20	14	22	24	184
	av.	4.0	4.0	2.7	3.2	3.1	3.2	2.8	2.9	2.4	2.5	2.2	2.84
	no.		1	35	23	21	17	14	10	7	21	27	140
	av.	3.0	1.0	2.1	2.5	2.2	2.1	2.2	2.4	2.0	2.3	1.6	2.14
	no.		1	28	19	16	15	7	8	2	6	9	82
♂	av.	2.0	2.0	0.9	1.4	1.8	1.5	1.9	1.5	1.5	1.8	0.4	1.47
	no.		2	26	20	15	11	11	10	11	15	9	102
	av.	1.0	2.0	0.5	0.7	0.6	0.9	0.8	0.6	0.6	0.7	0.9	0.71
	no.		3	10	12	12	1	11	6	3	10	4	59
	av.	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.5	0.0	0.3	0.3	0.20
	no.		18	47	37	23	21	20	16	13	16	5	151
	av.	4.0	2.7	2.4	1.8	1.4	1.4	1.7	1.4	1.0	1.3	1.4	1.49
	no.		14	39	25	18	21	16	7	10	14	12	123
	av.	3.0	2.1	1.6	1.0	0.7	0.8	0.7	1.1	1.4	0.9	0.9	0.90
	no.		14	37	30	30	24	24	13	11	11	6	149
♀	av.	2.0	1.4	0.8	0.5	0.5	0.5	0.6	0.5	0.4	0.2	0.0	0.47
	no.		11	31	23	24	21	15	9	6	8	3	109
	av.	1.0	0.3	0.2	0.3	0.3	0.3	0.2	0.1	0.2	0.0	0.3	0.24
	no.		7	15	14	9	3	2	4	5	5	5	47
	av.	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.05

the next pelage and thereafter remain about the same or, in the case of high grades, decline slightly. The females lose most of their dinginess in the first six or eight months but thereafter change little on the average. The grand averages of all grades after six months bring out the difference between the sexes in loss of dinginess.

A number of characters of the guinea pig, most notably the occurrence of polydactyly and the amount of color in the spotting pattern have been shown to be greatly affected by the age of the mother (less polydactyly and more

white among the progeny of mature than of immature females) (WRIGHT 1926). It is of some interest to see whether dinginess belongs in this group, especially as in this case the females lose their dinginess as they become mature which, on a Lamarckian view might be supposed to have a corresponding effect on the offspring. The average grades according to age of mother in the high grade derivatives of stock S and of experiments SP, SK and SA are shown in table 11.

TABLE 11

Relation of age of mother to average grade of dinginess of offspring in Stock S and from matings between high grades in experiments SP, SK and SA.

AGE OF MOTHER (MONTHS)	STOCK S		EXPERIMENTS SP, SK, SA	
	NO.	MEAN SE	NO.	MEAN SE
3–	100	2.91±.11	51	3.30±.13.
6–	82	2.90±.11	51	3.16±.15
9–	90	3.03±.10	58	3.14±.16
15–36	54	3.26±.11	28	3.46±.22

There are no consistent differences among the averages. In stock S, the distributions yield $\chi^2=9.4$, n=9, Probability, 0.30–0.50. In the other group $\chi^2=20.3$, n=12, Probability, 0.05–0.10.

Interactions with known color factors

Dinginess is not expressed in the white areas of the piebald pattern, due to the incomplete recessive *s*, but, as noted earlier, seems to be as strongly expressed in the brown spots of piebalds as in the corresponding areas of self browns.

Similarly dinginess is not expressed in self yellows (*ee*) or in the yellow hairs of tortoiseshells (e^pe^p, e^pe) but seems to be equally expressible in brown hairs of tortoiseshells and of self browns (*E*-).

Dinginess occurs in the brown of brown agoutis (*A*-) and according to EATON is more strongly developed than in nonagouti browns (*aa*). In my experiments, there have not been a sufficient number of litters with segregation of brown agoutis and browns for an adequate comparison.

The brown factor *b* is necessary, at least for more than a trace of dinginess. According to IBSEN, traces of white tipping may be present in blacks (*B*). I have not observed anything in black littermates of dingy browns that could be said with confidence to be the same condition but such litters have not been produced in adequate numbers. There is also, as noted, some doubt as to whether IBSEN'S white tipping is the same as the dinginess of brown in my colony.

The remaining three major series of alleles are concerned with the intensity of black, brown and yellow. Their interaction with each other in nondingy browns are shown in table 12 in which the amount of brown pigment ex-

tractible in genotype (*S E aabb*) *C-P-* is taken as 100 (WRIGHT 1942). The albino series (*C*, c^k, c^d, c^r, c^a) is represented by the three alleles that have been studied most intensively in connection with dinginess. Replacement of c^k by c^d or c^r gives somewhat smaller amounts of brown. *C* and *P* appear to be completely dominant and replacement of *F* by *f* in the presence of *P* appears to have no effect in nondingy browns. *F* is not completely dominant over *f* in pale browns, genotype *bbC-Ffpp* having only about 24 percent as much brown as *bbC-P-* instead of 30 percent as in *bbC-FFpp*. The very pale yellow or cream of *bbC-ffpp* seems to contain no brown.

TABLE 12

The appearance and amount of brown pigment relative to that with C-P- in combinations of C, c^k, c^a; F, f and P, p.

	(F, ff)P-	*FF pp*	*ff pp*
C-	Brown (100)	Pale Brown (30)	Pale Cream (0)
c^kc^k	Brown (96)	Pale Brown (25)	White (0)
c^kc^a	Brown (83)	Pale Brown (15)	White (0)
c^ac^a	White (0)	White (0)	White (0)

The effects of these genes on black (*B*) are somewhat parallel to those on brown. Replacement of either *C* or *F* by lower alleles reduces the intensity of yellow. Thus c^kc^k can easily be distinguished from *C*, and *ff* from *F* in tortoiseshells though not in self browns. Replacement of *B* by *b* or of *P* by *p* has no effect on intensity of yellow.

The observation that the occasional brown-yellow tortoiseshells($e^pe^pbbc^kc^kP$-*FF*) and pink eyed pale browns (*bbC-ppFF*), that segregated out in early generations of stock S, never showed the dinginess which was usually present in their brown-red littermates (e^pe^pC-*P-FF*), led to experiments (SK, SP) intended to test these apparent interaction effects more thoroughly. Another experiment (SF) was made to test whether dinginess could be introduced into brown-yellow tortoiseshells that owed their dilution of yellow to *ff* instead of to c^kc^k. As the interpretation of the results in the last case turned out to involve fewer complications these data will be considered first.

Effect of F, f

High grade dingies of stock S were mated reciprocally with brown-yellow tortoiseshells of a strain (F) in which at this time all animals were homozygous *CCffPP*. Most of the young (table 13) showed low grades of dinginess (average 1.5±0.1). There were no significant differences by direction of cross or sex of young (averages for 16(*DR*) ♀'s 1.6, for 25(*DR*) ♂'s 1.5, for 12 (*RD* ♀'s 1.2 and for 3(*RD*) ♂'s 1.3).

Backcrosses to stock F gave 41 young of which 24 had red spots (*Ff*), 16 had dilute yellow spots (*ff*) and one had no yellow spots and therefore might either be *Ff* or *ff*. Only seven of the 24 known to be *Ff* had any dinginess (average for *Ff* 0.4±0.1) and none of the 16 known to be *ff* were dingy.

The significant reduction of the average for *Ff* (1.5±.10 to 0.4±.13) on backcrossing to stock F shows that the latter must have introduced an independent factor or factors that inhibit dinginess. Results in later generations show that there is, in fact, no difficulty in obtaining *Ff* of grade 4. Brown-reds from *Ff*×*ff* in which both parents were half to three-quarter blood of stock S average 2.57±.13. This is significantly lower than *FF* of stock S but is higher than the average for Brown-reds from *Ff*×*Ff* and about the same

TABLE 13

The distribution of grades of dinginess in the offspring of matings involving only segregation of F, f among known factors. The ancestry (stock S, or F, F_1, ¾ blood stock S, etc.) is indicated in parentheses and is followed by the average grade.

DINGIER PARENT		LESS DINGY PARENT		MATINGS	GENOTYPE	0	1	2	3	4	NO.	AV.	SE
FF (*S*)	3.7	*ff* (*F*)	0.0	8	*Ff*	5	24	24	3		56	1.45	±.10
Ff (F_1)	2.2	*ff* (*F*)	0.0	5	*FF*	17	5	2			24	0.38	±.13
					?		1				1	1.00	
					ff	16					16	0.00	
FF (*S*)	4.0	*Ff* (F_1)	0.5	2	*F-*	1		5	2	3	11	2.55	±.37
Ff (F_1, (¾) *S*)	2.3	*F-* (*ca* (¾) *S*)	2.3	3	*F-*		1	7	1	3	12	2.50	±.29
FF (*ca* ½*S*)	3.4	*ff* (*ca* (¾) *S*)	0.8	7	*Ff*	5	9	13	7	2	36	1.78	±.18
FF (*S*)	3.6	*ff* (*ca* (¼)*S*)	1.2	8	*Ff*	2	7	20	8	3	40	2.08	±.15
Ff ((½)-(¾)*S*)	2.8	*ff* ((½)-(¾)*S*)	1.3	28	*Ff*	6	6	17	31	15	75	2.57	±.13
					?		3	4	1		8	1.75	
					ff	12	20	23	17	2	74	1.69	±.13
Ff ((½)-(¾)*S*)	2.6	*Ff* ((½)-(¾)*S*	1.6	24	*F-*	3	15	27	24	17	86	2.43	±.12
					?		1	5	3		9	2.22	
					ff	10	8	9	5	1	33	1.36	±.20
ff ((½)-(¾)*S*)	1.6	*ff* ((½)-(¾)*S*)	0.7	18	*ff*	11	42	35	9		97	1.43	±.08

amount of ancestry of stock S although about one-third of these should be *FF*. The data give no reliable evidence for any difference between *FF* and *Ff* where the rest of the genotype is the same.

The results in the later generations show that it is also possible to introduce dinginess into *ff*. But while a few have been given grade 4, it is clear that the average is systematically lower than for *Ff*. In the case of matings of type *Ff*×*ff* the young known (by their dilute yellow spotting) to be *ff* are lower than those known (by their red spotting) to be *Ff* by 0.88±.18. In the case of matings *Ff*×*Ff*, the brown-yellows (*ff*) are lower than the brown-reds (*Ff* or *FF*) by 1.07±.23. Those without yellow spotting and thus unclassifiable are intermediate in both cases. The average for young from *ff*×*ff* (extracted) is very low (1.43±.08) in view of the amount of *S* ancestry and thus confirms the effect of *ff* indicated by the preceding results.

The data from the backcrosses of F_1 to stocks F and S are not adequate for a decision on whether stock F differs from S by one incompletely recessive gene (other than *f*) or by multiple factors although the fact that the upper

50 percent of *Ff* in the backcross to stock F is below F_1 in average, while the lower 50 percent in the backcross to stock S is above F_1, suggests more than one factor. The small numbers are, however, compatible with the one factor interpretation.

It is noteworthy that the antagonistic effect of *ff* on dinginess is the first clear effect of *f* on dark pigmentation in the presence of *P*. It is also noteworthy that this effect, which is in the direction of intensification of brown, is in the opposite direction to the elimination of brown by *ff* in the combination *ffpp*.

Effect of P, p

Dingy browns of stock S were mated with pale browns (*C-ppFF* and c^kc^kppFF) from various sources as the first step in an attempt to introduce dinginess into this color. The young in F_1 (intense brown) consisted of 40 nondingy and only three with a trace of dinginess. In outcrosses of stock S to browns (nondingy or nearly so) from similar sources but known to be *Pp*, half the young showed some dinginess and two were of grade 4. F_2 from the first cross also included high grade dingies as well as ones of low grade and nondingies. The pink-eyed pale brown-red segregants (*C-pp*) lacked all trace of dinginess as did a few brown-yellow (c^kc^kP-) and pale brown-yellow (c^kc^kpp) segregants. Table 14 gives these results and those in later generations, except that the few animals shown to be c^kc^kP or c^kc^kpp by their yellow spotting are omitted. None of these showed any dinginess.

The results in F_1 and F_2 show that the pale brown parent must have introduced a gene or genes that tend to suppress dinginess, that there is segregation of high grade dinginess in F_2 and that dinginess is at least difficult to introduce into pale brown.

The complete absence of dinginess in the *pp* segregants of F_2 continued into later generations even when the amount of ancestry of stock S was increased to three-fourths or more. The attempt to obtain pale browns from high grade dingy parents, was, however, a failure. The brown young from matings that produced pale browns at all were moreover predominantly nondingy or of low grade. With one exception, matings in which either parent was of high grade (3, 4) produced no pale brown young. Matings in which either parent was *pp* produce only low grade dingies (and pale browns) except for one individual called grade 3. Nearly all matings in these experiments between animals of grades 0 or 1, or between such animals and pale brown produced some pale browns. All of the results point to the conclusion that *p* strongly reduces dinginess even in heterozygotes. Only three animals of grade 3 and none of grade 4 have yet been found which were *Pp*. One of these is shown in the table as coming from a class of matings of type $PP \times pp$. The second came from the same class but was born dead and so does not appear in the table. The third came from a mating of type $Pp \times Pp$ and was proved by a breeding test to be *Pp*. All of these were about three-fourths blood of stock S.

Linkage of *p* with a suppressor of dinginess is an alternative interpretation. The fact that *p* from all sources seems to have this effect and that nothing

suggesting crossingover has occurred in the course of the experiments, indicates that the effect is inherent in *p* rather than linked.

This conclusion was unexpected for two reasons. First there has been no indication from other experiments that *P* ever falls short of complete dominance over *p* even when other factors tend to reduce black or brown pigmentation to such a point that slight differences between *PP* and *Pp* should be easily

TABLE 14

Distribution of grades of dinginess among the offspring of matings involving p but not f. F_1 was largely from CCPP×c^kc^kpp. Some later matings produced yellow spotted young (c^kc^kP-, c^kc^kpp). These young are omitted. Ancestry and average grade of parents are indicated. PP? and Pp? refer to animals which may have been either PP or Pp by ancestry and descendants but are questionably assigned a genotype by grade. These were all (1/4) blood or more of stock S.

PARENT (HIGHER GRADE)			PARENT (LOWER GRADE)			NO. MATINGS	*pp*	GRADE OF OFFSPRING						NO. P-
								0	1	2	3	4	5	
PP	(*S*)	3.5	*pp*	(Misc.)	0.0	10	0	40	3					43
PP	(*S*)	3.1	*Pp*	(Misc.)	0.1	8	0	17	9	8		2		36
Pp	(F_1)	0.2	*Pp*	(F_1)	0.2	10	16	27	11	6	5	3		52
PP	((1/2)*S*)	2.9	*pp*	((1/2)*S*)	0.0	3	0	26	11	2				39
Pp	((1/2)*S*)	1.0	*pp*	((1/2)*S*)	0.0	4	4	3	1					4
Pp	((1/2)*S*)	1.4	*Pp*	((1/2)*S*)	0.8	6	11	16	4	7	6	3		36
PP	(*S*, (3/4)*S*)	3.1	*pp*	((1/2)*S*, (3/4)*S*)	0.0	9	0	18	35	20	1			74
Pp	(*ca* (3/4)*S*)	2.3	*pp*	((1/2)*S*, 3/4)*S*)	0.0	5	20	8	9	2				29
Pp	(*ca* (3/4)*S*)	1.3	*Pp*	(*ca* (3/4)*S*)	1.1	13	38	24	17	15	22	10	1	89
PP	(*S*, (3/4)*S*)	3.2	*Pp*	((1/2)*S*, (3/4)*S*)	0.8	5	0	18	8	7	8	4		45
PP?		3.5	*PP?*		3.3	8	0		1	12	18	25	8	64
PP?		3.6	*PP?*		2.0	4	0	1	3	9	10	20		43
PP?		2.0	*PP?*		2.0	2	0		1	4	5	7		17
PP?		3.8	*Pp*		2.0	4	0	6	1	4	2	3		16
PP?		3.6	*Pp?*		0.3	6	0	15	4	6	5	8		38
PP?		2.0	*Pp?*		0.3	3	0	12	6	7	4	3		32
P-		0.5	*Pp*		0.0	2	0	7	1	4				12
P-		1.0	*Pp?*		0.0	1	0	2		1		1		4

detected if present. Secondly, *Pp* gives a more intense brown than either *pp* or *PP*. In extreme dinginess of grade 5, *PP* may be even paler, though more irregular, than *pp*.

Effect of C series

The few brown-yellow tortoiseshells (c^kc^k) that segregated out in experiment S completely lacked dinginess and often appeared to be more intense in the brown parts of their coats than their dingy brown-red littermates. This called to mind IBSEN'S observation that white tipping could not be introduced into browns of genotype c^rc^r. As c^k is the closest of the albino alleles to *C* in its effects on color, an experiment (SK) was started with the purpose of introducing dinginess into c^kc^k browns if possible. Table 15 gives the data from this experiment excluding all matings in which either parent was definitely

known from its ancestry or descendants to be *Pp*. Unfortunately this excludes all of F_1 and most of the early matings as the effect of *Pp* on dinginess was not anticipated. Some of the matings included may involve *Pp* and this may account for some of the young of low grade.

The results resemble those with *P*, *p* in that no clear case of dinginess has been observed in the recessive segregants, here brown-yellow tortoiseshells (c^kc^k). In two or three cases there was some suggestion of dinginess on the cheeks but not enough to be sure that this was not a manifestation of the tor-

TABLE 15

Distribution of grades of dinginess among offspring of matings involving C, c^k but not F, f. Matings (including all of F_1) in which either parent was known to be either Pp or pp are excluded. Matings of type $Cc^k \times Cc^k$ are divided into a group in which both parents were of high grade (3, 4) and one in which one parent was of grade 2 and the other 2 or 3. In all cases both parents are 50% or more of stock S. The average grades of higher and lower parents in the matings in each group are shown.

PARENT (HIGHER GRADE)		PARENT (LOWER GRADE)		NO. MATINGS	GENOTYPE	GRADE OF OFFSPRING					NO.	AV.
						0	1	2	3	4		
CC	3.9	c^kc^k	0	5	Cc^k	9	7	2	13	6	42	2.00
Cc^k	2.6	c^kc^k	0	14	Cc^k	1	7	11	8	9	36	2.47
					?	4	1	2			7	1.57
					c^kc^k	35					35	0.00
Cc^k	2.2	Cc^k	2.0	7	*C*-	4	1	8	7	6	26	2.38
					?			1	2	1	4	3.00
					c^kc^k	17					17	0.00
Cc^k	3.8	Cc^k	3.6	7	*C*-		1	9	4	20	34	3.26
					?			1	2	1	4	3.00
					c^kc^k	14					14	0.00

toiseshell pattern. The results differ from those with *P*, *p* in that high grade parents often turned out to be heterozygous (Cc^k) as shown by the production of brown-yellow young. In such cases, moreover, the brown-red young were predominantly of high grade. Again matings between a c^kc^k parent and either *CC* or Cc^k produced many high grade dingies.

These results suggest that Cc^k does not differ from *CC* in relation to dinginess. The average grades for the brown-red young are, however, rather low for the amount of ancestry of strain S (one-half to three-fourths). Moreover the average grade of brown-reds from matings of type $Cc^k \times Cc^k$ which should include 33 percent homozygotes is higher (2.90) than the average from matings which can not produce homozygotes (2.20). This comparison does not, however, have the validity of a comparison between classes of young from the same matings. It has not been possible to obtain a decisive answer to this question by more detailed analysis of these data.

In another experiment (SA) an attempt was made to produce albinos (c^ac^a) capable of transmitting dinginess. Table 16 gives a condensed account of the results. Again no matings are included in which either parent was definitely

known to be other than *PP* from ancestry or descendants. Unfortunately the available brown eared albinos (*EEaabbc^{a}c^{a}P-FF*) all had *p* in their recent ancestry and some of them may well have been *Pp* and again this could be responsible for some of the low grade young.

The average for F_1 (stock S by the foundation albinos) was low (1.36) and included none of grade 4. In later generations, however, with more ancestry of stock S, young were obtained from matings of type $CC \times c^a c^a$ and $Cc^a \times c^a c^a$

TABLE 16

Distribution of grades of dinginess among offspring of matings involving C, c^a but not F, f. Parents known to be Pp or pp are excluded. In all except the first row both parents are 50% or more of stock S. The average grade of higher and lower parent in the matings of each group are shown.

PARENT (HIGHER GRADE)		PARENT (LOWER GRADE)		NO. MATINGS	$c^a c^a$	GRADE OF C OFFSPRING					NO. C-	AV. C-
						0	1	2	3	4		
CC (S)	3.4	$c^a c^a$ (EBA)		5	0	15	14	19	8		56	1.36
CC	3.4	$c^a c^a$		7	0	8	17	21	13	2	61	1.74
Cc^a	2.6	$c^a c^a$		8	44	1	3	17	16	6	43	2.53
Cc^a	2.1	Cc^a	1.5	17	46	29	20	41	22	10	122	1.70
CC (S)	4.0	Cc^a	1.1	3	0		2	8	4	4	18	2.56

that were of grade 4. Thus Cc^a, like Cc^k, can not have as great a suppressive effect on dinginess as does *Pp*. The rather low averages throughout in comparison with stock S suggest, however, that Cc^a may have some effect but the average is no higher in the progenies that include *CC* than in those that do not.

No systematic attempt has been made to introduce dinginess into browns with other combinations of albino alleles. The following have, however, segregated out of matings in progenies that contained red spotted dingies (*C-*): 12 $c^k c^r$, 24 $c^k c^a$, 14 $c^d c^a$, 6 $c^r c^r$, 3 $c^r c^a$. The young in the first three of these classes were identifiable by cream spotting (and by ancestry), the last two by white spotting or silvering of the synthetic type (and by ancestry). No trace of dinginess was observed in the brown of any of these. It seems safe to conclude that gene *C* is necessary for the expression of dinginess.

Joint effects of P, p and the C series

Genes c^k and c^a and to a less extend c^d and c^r were present in experiment SP and it is possible that some of the suppression of dinginess attributed to *Pp* may have been due to joint effects of *Pp* and heterozygosis in the C-series. An enumeration (table 17) of known double heterozygotes yields the following in which c^s here refers to cases in which it was merely known that a lower albino allele was present.

It is noteworthy that 82 percent of the known double heterozygotes were completely lacking in dinginess and none of the rest showed more than a trace. Thirty-six of these were from the first cross between stock S and unrelated ani-

mals and might owe their low grade to such a factor or factors as was indicated to be present in stock F. The remaining 21, however, had *S* ancestry, usually 50 percent or more, on both sides.

TABLE 17

Distribution of grades among individuals known to be Pp and also heterozygotes of C with a lower allele.

	F_1(S×Misc.) GRADE		BOTH PARENTS WITH S ANCESTRY GRADE	
	0	1	0	1
Cc^kPp	17	2	6	2
Cc^rPp	—	—	5	0
Cc^aPp	—	—	5	3
Cc^zPp	14	3	—	—
	—	—	—	—
Total	31	5	16	5

Effect of age on different genotypes

The average for all grades after six months of age was found for each mated animal. Table 18 shows the relation between initial grade and the average for each genotype and sex after six months. Genotype *C-P-FF* includes all animals known to be carrying *C* and *P* and not known from ancestry or descendants to carry *p* or any lower allele of *C* (though heterozygosis may be suspected because of grade in some cases).

The initial grades of these mated animals agree fairly well with those based on the progeny in the preceding discussion.

The grades after six months show the reduction previously referred to, but otherwise tend to parallel the grades at weaning. It is, however, noteworthy that Cc^kP-*FF* and Cc^aP-*FF* in both males and females show a much greater loss of dinginess than does *CCPPFf* although the initial grades differ but little. These adult grades give more reliable evidence for effects of Cc^k and Cc^a than obtained from the initial grades. The very low grades of *CCPPff*, *C-PpFF* confirm the previous conclusions and the exceptionally low grade of Cc^zPpFF in males supports the view that Cc^z and *Pp* act cumulatively.

The effects of unrecognized *Pp* (in *P*-) and of residual factors can be largely eliminated by confining attention to animals that were of grades 3, 4 or 5 initially. This is done in table 19. The group *C-PPFF* is subdivided into those from stock S, experiment SP, and experiments SK or SA.

The initial grades mean nothing here because of the selection, which is more drastic in *CCPPFf* and Cc^zPPFF than for *C-PPFF*. The grades after six months bring out clearly the tendency of Cc^zPPFF to lose dinginess more than does *C-PPFF* from the same experiments. It appears indeed that *C-PPFF* from SK and SA has been selected to an even higher level of dinginess than in

TABLE 18

Average grade after 6 months of age of animals of indicated genotypes and each initial grade. The class ♂ C-P-FF initial grade 4 includes 2 of initial grade 5 which averaged 3.70 after 6 months.

GENOTYPE	INITIAL GRADE 0 NO.	0 AV.	1 NO.	1 AV.	2 NO.	2 AV.	3 NO.	3 AV.	4 NO.	4 AV.	TOTAL NO.	INITIAL GRADE	AFTER 6 MONTHS
♂ *C-P-FF*	1	0.00	4	0.73	4	2.60	21	2.97	43	3.28	73	3.41	2.97±.13
♂ *CCPPFf*	1	0.00	3	0.50	11	2.02	6	2.90	7	3.29	28	2.54	2.30±.22
♂ *C-PPff*	9	0.28	6	0.67	6	1.25	—		1	3.70	22	1.00	0.80±.25
♂ *Cᵗ P-FF*	1	0.00	2	2.15	5	0.78	6	1.08	10	1.91	24	2.92	1.41±.18
♂ *Cᵉ P-FF*	2	0.00	6	0.73	5	1.30	8	1.25	1	4.00	22	2.00	1.13±.23
♂ *C-PpFF*	3	0.37	6	0.25	4	1.30	1	1.50	—		14	1.21	0.66±.19
♂ *Cᵗ PpFF*	3	0.17	2	0.20	—		—				9	0.44	0.18±.09
♂ *Cᵉ PpFF*	2	0.35	2	0.00									
♀ *C-P-FF*	6	0.00	7	0.50	15	0.83	21	1.26	46	1.73	95	2.99	1.29±.11
♀ *CCPPFf*	2	0.00	5	0.20	8	0.59	7	1.69	4	2.20	26	2.23	1.01±.20
♀ *C-PPff*	4	0.00	6	0.17	11	0.68	4	1.00			25	1.60	0.50±.15
♀ *Cᵗ P-FF*	1	0.00	3	0.17	7	0.23	5	0.36	6	0.82	22	2.55	0.40±.09
♀ *Cᵉ P-FF*	2	0.50	9	0.24	12	0.37	7	0.60	3	1.70	33	2.00	0.51±.11
♀ *C-PpFF*	3	0.03	5	0.04	19	0.10	—		—		19	1.11	0.10±.04
♀ *Cᵗ PpFF*	2	0.00	2	0.50	—		—		—		8	0.38	0.13±.08
♀ *Cᵉ PpFF*	3	0.00	1	0.10	—		—		—				

stock S. The difference between *C-* and *Cᵉ* in males in experiments SK, SA alone is 2.29±.30. Using all males of genotype *C-PPFF* the difference is 1.60±.23 and in females .83±.18, highly significant in spite of the great reduction in grade in all females. Genotype *Ff* of initial grade 3 or 4 does not, on the other hand, differ significantly from *FF* after six months, either in males or females.

TABLE 19

Average grade after 6 months of age of animals of the indicated genotypes and of grade 3 or more initially. The group C-PPFF is divided into 3 classes according to source.

GENOTYPE	NO.	INITIAL GRADE	AFTER 6 MONTHS
♂ *C-PPFF*(S)	39	3.72	3.05±.14
♂ *C-PPFF*(SP)	12	3.58	2.85±.30
♂ *C-PPFF*(SK, SA)	13	3.77	3.87±.23
♂ *CCPPFf*(SF)	13	3.54	3.11±.19
♂ *Cᵉ PPFF*(SK, SA)	25	3.44	1.58±.20
♀ *C-PPFF*(S)	46	3.70	1.57±.15
♀ *C-PPFF*(SP)	13	3.54	1.51±.30
♀ *C-PPFF*(SK, SA)	8	3.88	1.84±.51
♀ *CCPPFf*(SF)	11	3.36	1.87±.31
♀ *Cᵉ PPFF*(SK, SA)	21	3.43	0.76±.13

The only evidence for an effect of *Ff* is in the unselected initial grades and here the undoubted presence of unfavorable residual heredity in many *Ff* and the likelihood that *Pp* is present in some of those called *C-P-FF* make comparisons unreliable.

Developmental Factors

To some extent at least dinginess tends to develop most in the regions of the body (head and back as opposed to belly) and of individual hairs (subterminal region) in which brown would otherwise be most intense. Apparently there is a certain optimum in the complex of factors of all sorts that control the intensity of brown. Either excess or defect in this complex results in less than maximum intensity. The actual amount of brown pigment in dingy hair has not yet, however, been investigated.

SUMMARY

Four types of silvering of the coat of the guinea pig are discussed.

There is a stationary type in which white or light colored hairs are mingled with those of the color determined by the rest of the genotype. It is usually restricted to the belly in lower grades but in higher grades appears also on the back. The head and legs are least affected. Silvering depends in the main on a single incompletely recessive autosomal gene (*si*) but varies continuously over a wide range because of minor factors, genetic and non-genetic.

There is a synthetic type in which the tortoiseshell pattern of mingled dark and yellow hairs is reduced to a pattern, sometimes simulating the preceding, by action of genotypes which reduce yellow disproportionately. The genetics of this type is well known.

There is a progressive type of silvering or grizzling which appears only after the first pelage. White hairs appear first on the posterior back but become sprinkled later over the entire back, head, and belly, among hairs of the color determined by the rest of the genotype. This depends on an incompletely recessive autosomal gene (*gr*), in confirmation of LAMBERT.

There is a retrogressive type of silvering or dinginess which is manifested only in otherwise intense browns (*bb C P*). In the lowest grade there are scattered hairs on cheeks and nape in which there is subterminal banding with pale brown. In higher grades, such hairs are sprinkled over the whole head and back, and in the highest practically all hairs are affected. Dinginess is reduced with age, especially in females. At least four series of alleles are involved. One or more autosomal factors, again incompletely recessive, are necessary for any dinginess. If these are favorable, all known series of alleles, that affect intensity of brown in any combination, tend to modify dinginess, but in the opposite direction from that which would be expected from their ordinary effects. *CCPPF-* is necessary for the highest grade of dinginess. Cc^z($=Cc^k$, Cc^d, Cc^r or Cc^a) reduces dinginess, at least in mature animals. No dinginess appears in combinations lacking $C(c^kc^k$, etc.). *Pp* reduces dinginess more than does Cc^z and acts cumulatively with the latter. There is no dinginess in pale browns (*pp*). It is uncertain whether *Ff* affects dinginess, but *ff* reduces it considera-

bly. To some extent at least dinginess tends to appear most in the region of the body and of the hairs in which brown would otherwise be most intense.

The factors that affect dinginess give good illustrations of the principle that gene replacements that increase development of a character in certain combinations may reduce development in others.

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GENETICS OF NATURAL POPULATIONS. XIV. A RESPONSE OF CERTAIN GENE ARRANGEMENTS IN THE THIRD CHROMOSOME OF *DROSOPHILA PSEUDOOBSCURA* TO NATURAL SELECTION

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INTRODUCTION

CYCLIC changes in the frequencies of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* take place in populations of some localities on Mount San Jacinto, in California (DOBZHANSKY 1943). Chromosomes with Standard gene arrangement (ST) are least frequent in June, increase in frequency during the summer, remain at a high level during autumn and winter, and decline during spring. Chromosomes with Arrowhead (AR) and particularly with Chiricahua (CH) gene arrangements undergo cycles opposite in sign to that of ST chromosomes. The supposition that these cyclic changes are due to natural selection is in accord with all the facts available, and constitutes a fruitful working hypothesis for further research.

Artificial models of natural populations can be created with the aid of population cages of the type first proposed by L'HÉRITIER and TEISSIER (see WRIGHT and DOBZHANSKY 1946 for a description of the model used). If a mixture of flies containing more than 30 percent Chiricahua and less than 70 percent of Standard chromosomes is introduced in a population cage, the frequency of ST rises and that of CH decreases generation after generation. Yet, the CH chromosomes are never eliminated completely; the population reaches an equilibrium at about 70 percent ST and 30 percent CH. If the initial population contains many CH and few AR chromosomes, the frequency of AR increases at the expense of CH until an equilibrium is established. These changes in the frequencies of the chromosomal types take place in population cages kept at temperatures above 20°C; at 16½°C no changes are observed.

The simplest hypothesis that fits the observations on the behavior of the chromosomal types in population cages is that, at temperatures above 20°C, the heterozygotes ST/CH have a higher adaptive value than ST/ST homozygotes, and these latter higher than CH/CH homozygotes. Similarly, the adaptive values of the carriers of AR and CH gene arrangements form the series AR/CH > AR/AR > CH/CH. At 16½°C the adaptive values of the homo- and heterozygotes are similar, at any rate more so than they are at higher temperatures (WRIGHT and DOBZHANSKY 1946).

However, much remains to be learned about the operation of natural selection which brings about the changes both in free-living populations and in the experimental cages. Nothing is known at present about the stage of the life

cycle of the fly at which the differential survival of the different chromosomal types take place. It is not certain that selection acts equally on females and males, or that the selection intensity remains constant regardless of the frequencies of the chromosomal types in the population (WRIGHT and DOBZHANSKY 1946). The present article reports the results of experiments designed to test further the validity of the hypothesis of natural selection in the case of the chromosomal variation in *D. pseudoobscura*, as well as to approach the more special problems just stated.

MATERIAL AND METHODS

Except when otherwise specified, the progenitors of all the flies used in this study were collected at Piñon Flats, San Jacinto, California. The collections were made partly in 1942 by the writer, but mostly in late April of 1945 by MR. ALEXANDER SOKOLOFF.

The technique of preparing the initial mixtures of flies with desired proportion of chromosomes of different kinds has been described by WRIGHT and DOBZHANSKY (1946). The same paper contains descriptions of the methods of sampling the composition of a population in an experimental cage and of cytological examination of the chromosomes.

CHANGES OBSERVED IN THE POPULATION CAGES NUMBERS 22 AND 23

On September 19, 1945, an initial population of 1366 flies was introduced in cage number 22, which was then placed in a room with constant temperature regulated at 25°C and with humidity of about 70 percent. This population contained 20.31 percent of ST and 79.69 percent of CH chromosomes. The ST chromosomes came from 20 different strains and CH chromosomes also from 20 different strains (see WRIGHT and DOBZHANSKY 1946, for comment on the importance of using several strains as source material for population cage experiments). On the same day, 1763 flies containing a mixture of 20.72 percent AR and 79.28 percent CH chromosomes were placed in cage number 23. The AR chromosomes came from 11, and CH chromosomes from 15 different strains. Cage number 23 was placed in the same constant temperature and humidity room in which cage number 22 stood.

Samples of eggs deposited in the population cages were taken in late October, late November, and late December by the usual method (WRIGHT and DOBZHANSKY 1946), and the gene arrangement was determined in 300 chromosomes in each sample. The results are summarized in table 1. In early January of 1946 both cages developed mite infections and were destroyed.

Table 1 shows that in cage number 22 the frequency of ST chromosomes rose from the initial 20.3 percent in September to 54.7 percent in December, and the frequency of CH dropped from 79.7 in September to 45.3 in December. Similarly, in cage number 23 there was an increase of the frequency of AR from 20.7 to 56.7 percent, and a decline of CH from 79.3 to 43.3 percent. There is no question that these changes are statistically significant; in fact the increases and decreases observed on successive months are also significant.

The changes observed in cages numbers 22 and 23 are in accord with the findings of WRIGHT and DOBZHANSKY (1946) in analogous experiments. The simplest hypothesis that fits both series of experiments is that, if the adaptive value of the heterozygotes ST/CH is taken to be 1.0, then the adaptive values of ST/ST and CH/CH homozygotes are, under the condition of the population cages, about 0.7 and 0.3 respectively. Accepting these values (slightly different

TABLE 1

Percentage frequencies of the different gene arrangements in the populations of the cages Nos. 22 and 23 at different times.

TIME	EXPERIMENT NO. 22			EXPERIMENT NO. 23		
	ST	CH	n	AR	CH	n
Initial (September)	20.3	79.7		20.7	79.3	
October	32.3	67.7	300	33.7	66.3	300
November	42.7	57.3	300	46.3	53.7	300
December	54.7	45.3	300	56.7	43.3	300

estimates, based on all the available data, will be found on p. 152), and taking the duration of a generation to be one month (which is a slight overestimate), one can easily compute the expected frequencies of ST in cage number 22 on different months. A comparison of the observed and expected frequencies is as follows (in percentages):

	OBSERVED	EXPECTED
September	20.3	20.3
October	32.3	35.1
November	42.7	47.0
December	54.7	54.8

The agreement is excellent. No estimates of the adaptive values of the genotypes AR/CH, AR/AR, and CH/CH in cages containing only these two gene arrangements have been made by WRIGHT and DOBZHANSKY. However, the parallelism between the changes in cages numbers 22 and 23 is so great (table 1) that the relative adaptive values of these three genotypes are probably not far from those of ST/CH, ST/ST, and CH/CH (see above). The population in cage number 22 should eventually reach the equilibrium values of about 70 percent ST and 30 percent CH chromosomes, after which the changes should no longer occur. The mite infection made it impossible to check the validity of this prediction in cage number 22; however, it has been realized in analogous experments of WRIGHT and DOBZHANSKY (1946), as well as in experiments to be reported on elsewhere.

THE HARDY-WEINBERG PROPORTIONS IN EGG SAMPLES

Provided that the flies carrying different chromosomal types interbreed at random, the zygotes produced by a panmictic natural population, or by one

confined in a population cage, must contain heterozygotes and homozygotes in proportions indicated by the formula given about forty years ago by HARDY and WEINBERG. Taking the frequency of gametes carrying ST chromosomes to be q, and of gametes with CH chromosomes to be $(1-q)$, the frequencies of the homo- and heterozygotes will be as follows:

$$q^2\ \mathrm{ST/ST}:2q(1-q)\ \mathrm{ST/CH}:(1-q)^2\ \mathrm{CH/CH}$$

However, if the different classes of zygotes survive at different rates, their proportions may deviate from the ones demanded by HARDY-WEINBERG's formula. Thus, assuming the survival rates of the ST/ST, ST/CH, and CH/CH genotypes to be W_1, W_2, and W_3, the proportions of the different genotypes at the stage of the life cycle following that at which the selective elimination occurs will be:

ST/ST	q^2W_1
ST/CH	$2q(1-q)W_2$
CH/CH	$(1-q)^2W_3$

Disturbances of the HARDY-WEINBERG proportions may then be used to detect the occurrence of selective elimination of zygotes.

The composition of the populations in cages numbers 22 and 23, as well as in the experiments described by WRIGHT and DOBZHANSKY (1946), was examined at approximately monthly intervals. Every month pieces of the culture medium with eggs deposited on it were withdrawn from each population cage on six successive days. These eggs were placed in regular culture bottles and allowed to develop at room temperature; when small larvae appeared in the bottles they were given extra yeast to attain optimal nutrition. The salivary gland cells of 25 larvae from each culture were examined under a microscope. A monthly sample, consisting of six subsamples, includes therefore 150 larvae. The gene arrangements were determined in two chromosomes in each larva, heterozygous as well as homozygous individuals being easily distinguishable by presence of inversion loops or by the patterns of stainable discs in the chromosomes. Numbers of hetero- and homozygotes observed in all experiments involving ST and CH chromosomes are summarized in table 2, and in experiments involving AR and CH chromosomes in table 3. Gametic frequencies of the gene arrangements, that is the frequencies of each gene arrangement in the sample of gametes which gave rise to a given group of 150 larvae, can easily be determined. Each homozygote is formed, of course, by two gametes with identical chromosomes, while a heterozygote is a product of the union of two gametes with different chromosomes. A sample of 150 larvae came from 300 gametes with 300 third chromosomes. The gametic frequencies, expressed in percentages, are shown in the two rightmost columns in tables 2 and 3. Knowing the gametic frequencies one can compute, with the aid of the HARDY-WEINBERG formula, the numbers of hetero- and homozygotes expected to occur in each sample of 150 larvae. These computations involve the assumptions that the populations of the experimental cages are panmictic, and that the different classes of zygotes suffer no differential mortality between the egg stage and the

TABLE 2

Zygotic and gametic frequencies of the Standard (ST) and Chiricahua (CH) gene arrangements among the eggs deposited in the population cages.

CAGE NO.	DATE OF SAMPLE		ZYGOTIC FREQUENCIES			χ^2	GAMETIC FREQUENCIES IN PERCENT	
			ST/ST	ST/CH	CH/CH		ST	CH
		Obs	37	82	31		52.0	48.0
8	January	1944 Exp	40.5	74.9	34.5	1.33		
		Diff	−3.5	+ 7.1	−3.5			
		Obs	46	89	15		60.3	39.7
8	February	1944 Exp	54.6	71.8	23.6	8.61		
		Diff	−8.6	+17.2	−8.6			
		Obs	58	74	18		63.3	36.7
8	March	1944 Exp	60.1	69.7	20.2	0.58		
		Diff	−2.1	+ 4.3	−2.2			
		Obs	70	68	12		69.3	30.7
8	April	1944 Exp	71.4	64.2	14.4	0.65		
		Diff	−1.4	+ 3.8	−2.4			
		Obs	52	81	17		61.7	38.3
11	July	1944 Exp	57.1	70.9	22.0	2.99		
		Diff	−5.1	+10.1	−5.0			
		Obs	63	73	14		66.3	33.7
11	August	1944 Exp	65.9	67.0	17.0	1.20		
		Diff	−2.9	+ 6.0	−3.0			
		Obs	66	59	25		63.7	36.3
11	September	1944 Exp	60.9	69.4	19.8	3.36		
		Diff	+5.1	−10.3	+5.2			
		Obs	39	86	25		54.7	45.3
13	August	1944 Exp	44.9	74.3	30.8	3.65		
		Diff	−5.9	+11.7	−5.8			
		Obs	51	73	26		58.3	41.7
13	September	1944 Exp	51.0	72.9	26.1	0.00		
		Diff	0.0	+ 0.1	−0.1			
		Obs	41	77	32		53.0	47.0
19	December	1944 Exp	42.1	74.7	33.1	0.10		
		Diff	−1.1	+ 2.3	−1.1			
		Obs	56	78	16		63.3	36.7
19	January	1945 Exp	60.1	69.8	20.2	2.11		
		Diff	−4.1	+ 8.2	−4.2			
		Obs	53	75	22		60.3	39.7
19	February	1945 Exp	54.6	71.8	23.7	0.31		
		Diff	−1.6	+3.2	−1.7			
		Obs	65	66	19		65.3	34.7
19	March	1945 Exp	63.9	68.0	18.0	0.14		
		Diff	+1.1	− 2.0	+1.0			
		Obs	61	74	15		65.3	34.7
19	April	1945 Exp	64.0	68.0	18.0	1.17		
		Diff	−3.0	+ 6.0	−3.0			
		Obs	58	60	7		70.4	29.6
19	June	1945 Exp	62.0	52.1	11.0	2.94		
		Diff	−4.0	+ 7.9	−4.0			
		Obs	20	88	42		42.7	57.3
22	November	1945 Exp	27.3	73.4	49.3	5.93		
		Diff	−7.3	+14.6	−7.3			
		Obs	41	82	27		54.7	45.3
22	December	1945 Exp	44.8	74.4	30.8	1.57		
		Diff	−3.8	+ 7.6	−3.8			

TABLE 3

Zygotic and gametic frequencies of the Arrowhead (AR) and Chiricahua (CH) gene arrangements among the eggs deposited in the population cages.

CAGE NO.	DATE OF SAMPLE		ZYGOTIC FREQUENCIES AR/AR	AR/CH	CH/CH	χ^2	GAMETIC FREQUENCIES IN PERCENT ST	CH
		Obs	20	86	44		42.0	58.0
15	September 1944	Exp	26.5	73.1	50.5	4.71		
		Diff	−6.5	+12.9	−6.5			
		Obs	26	77	47		43.0	57.0
15	August 1944	Exp	27.7	73.5	48.7	0.34		
		Diff	−1.7	+3.5	−1.7			
		Obs	29	81	40		46.3	53.7
23	November 1945	Exp	32.2	74.6	43.2	1.04		
		Diff	−3.2	+6.4	−3.2			
		Obs	48	74	28		56.7	43.3
23	December 1945	Exp	48.2	73.6	28.1	0.02		
		Diff	−0.2	+ 0.4	−0.1			

stage of grownup larva at which the cytological examination is made. The expected numbers of homo- and heterozygotes, and the differences between observed and expected numbers, are shown in tables 2 and 3. Chi-squares that measure the statistical significance of these differences are also included in these tables. Each chi-square has one degree of freedom; this is because the gametic frequencies are calculated from the same observed zygotic data which are subsequently used for comparison with the zygotic frequencies expected if the HARDY-WEINBERG proportions are realized.

Among the twenty-one samples tabulated in tables 2 and 3, only one, that for February 1944 in cage number 8, shows a very significant difference between the observed and the expected frequencies of the homo- and heterozygotes. The chi-square is in this case equal to 8.61, which has a probability of chance occurrence of about 0.004. Two more samples, namely for November 1945 in cage number 22 and for September 1944 in cage number 15, show chi-squares of 5.93 and 4.71 respectively, which are ordinarily taken as indicating significant deviations between the observed and the expected figures. In all other samples the observed and expected numbers agree well enough.

However, it may be noted that among the twenty-one samples listed in tables 2 and 3 only two samples (September 1944 in cage 11 and March 1945 in cage 19) show numbers of homozygotes in excess, and numbers of heterozygotes, below the expectation. The remaining nineteen samples show greater or smaller excesses of heterozygotes and deficiencies of homozygotes. The total chi-square

for all data in table 2 is 36.6, which, for 17 degrees of freedom, has the probability of chance occurrence less than 0.004. There is consequently little doubt that the inversion homozygotes are discriminated against, and heterozygotes are favored, even among larvae raised on abundant food in culture bottles. Nevertheless, the conclusion to be drawn from the data in tables 2 and 3 is that the HARDY-WEINBERG ratios are approached rather closely. In any case, there is nothing in the data to indicate that the adaptive value of ST/ST zygotes is 70 percent, and of CH/CH zygotes only 30 percent, of the value of ST/CH zygotes, as postulated by WRIGHT and DOBZHANSKY (1946). Taking the data in table 2 as being representative of the conditions which obtain when the larvae from the eggs deposited in population cages are grown in regular culture bottles, PROFESSOR SEWALL WRIGHT has very kindly communicated to the writer the following estimates of the relative viabilities of the ST/ST, ST/CH, and CH/CH classes:

	W
ST/ST	0.907
ST/CH	1.000
CH/CH	0.782

From the data in table 3, the corresponding values for the AR/AR, AR/CH, and CH/CH zygotes are as follows:

	W
AR/AR	0.916
AR/CH	1.000
CH/CH	0.803

This is very far from the values 0.7, 1.0, and 0.3, for ST/ST, ST/CH, and CH/CH zygotes respectively, arrived at by WRIGHT and DOBZHANSKY on the basis of analysis of changes in the composition of the cage populations. The discrepancy may be explained in several ways. First of all, the hypothesis of WRIGHT and DOBZHANSKY does not postulate that the differences in the adaptive values manifest themselves through selective mortality between the egg stage when the samples are taken in population cages and the late larval stage when the examination of the chromosomes in the salivary glands is made. It is conceivable that the selective mortality occurs in the pupa stage, or that ST/ST and CH/CH flies have a lower longevity, or lower fecundity, or lower sexual activity than the adult ST/CH individuals. If so, the HARDY-WEINBERG proportions may be realized in the samples of eggs taken in the population cages, and yet the selective process may occur at later stages. Furthermore, selective mortality of larvae of the different genotypes may take place under the conditions of crowding and acute competition for food which prevail in the population cages, and not under the relatively more favorable conditions deliberately maintained in the culture bottles used for raising larvae intended for examination of the salivary gland chromosomes. Indeed, thousands of eggs are deposited in the population cages on the food in every jar inserted into a cage, but at best only hundreds of adult flies hatch in each jar. In a few days after the insertion of a fresh jar, the culture medium in population cages is

completely filled with young larvae most of which doubtless die before reaching full growth. The larvae that do grow up contain salivary chromosomes which are very unfavorable for cytological study. The technique of raising the larvae under optimal conditions is intended to make the cytological study easier. But by placing the larvae in near optimal conditions the differential mortality may well be diminished or even avoided. The fact that the composition of the population in the population cages changes very rapidly at 25°C, but not at 16½°C (WRIGHT and DOBZHANSKY 1946), shows that the differences in adaptive value between the chromosomal types are quite sensitive to environmental influences. Accordingly, a study of the HARDY-WEINBERG ratios was undertaken in flies that had developed in the population cages.

THE HARDY-WEINBERG RATIOS AMONG ADULT FLIES

On December 14, 1945, the adult flies present at that time in the population cages numbers 22 and 23 were transferred to bottles and etherized. Slightly more than one hundred males were chosen at random from amongst the flies in each cage. The males from cage number 22 were outcrossed singly to females known to be homozygous for ST chromosomes; the males from cage number 23 were likewise outcrossed to known AR homozygotes. The outcrosses were made in regular culture bottles, which were treated so as to insure an optimal development of larvae desired for cytological examination. When the larvae reached maturity, the salivary glands of six larvae from each bottle were stained in acetic orcein and examined for the condition of the third chromosomes. When two types of chromosomal structure (for example, ST/ST and ST/CH) are found among the larvae, it necessarily follows that the father of these larvae was a heterozygote carrying a ST and a CH chromosome. When all six larvae showed the same configuration in the third chromosome, the father was probably a structural homozygote. However, with six larvae studied there is still one chance in thirty-two that the father was a heterozygote but that by accident all the larvae examined were of the same chromosomal type. The observed numbers of the different chromosomal types among the males developed in the population cages are reported in tables 4 and 5 in the horizontal row labelled "old ♂♂."

On the same day, three jars containing mature ready-to-hatch pupae were withdrawn from the population cages numbers 22 and 23. The flies that hatched in these jars were collected for several days, males and females being separated so that the latter remained virgin. The flies from cage number 22 were then outcrossed singly to known ST/ST homozygotes, and those from cage number 23 to known AR/AR homozygotes. The larvae coming from the outcrosses were kept well fed, and when they matured six larvae from each cross were dissected and their salivary glands were stained in acetic orcein and examined under a microscope. Thus the zygotic chromosomal constitutions of the females and males that had developed in the population cages up to the stage of late pupa became known. The data are shown in tables 4 and 5 in the rows labelled "Young ♀♀" and "Young ♂♂."

The gametic frequencies of the gene arrangements in the samples of adult flies having developed in the population cages can easily be calculated from the zygotic data. The expected zygotic frequencies are then computed with the aid of the HARDY-WEINBERG formula. The expected numbers, and their deviations from the observed ones, are given in tables 4 and 5. Chi-squares are calculated to evaluate the statistical significance of the deviations observed. These chi-squares have one degree of freedom.

TABLE 4

Genetic structure of adult flies in population cage No. 22.

SEX AND AGE		ZYGOTIC FREQUENCIES			χ^2	GAMETIC FREQUENCIES IN PERCENT	
		ST/ST	ST/CH	CH/CH		ST	CH
Young ♀♀	Observed	31	83	16		55.8	44.2
	Expected	40.4	64.1	25.4			
	Difference	− 9.4	+18.9	− 9.4	11.24		
Old ♂♂	Observed	26	86	13		55.2	44.8
	Expected	38.1	61.6	25.1			
	Difference	−12.1	+24.2	−12.1	19.14		
Total	Observed	57	169	29		55.5	44.5
	Expected	78.5	126.0	50.5			
	Difference	−21.5	+43.0	−21.5	29.70		

The heterozygotes ST/CH are decidedly more frequent, and the homozygotes ST/ST and CH/CH less frequent, than expected. This is true for the male as well as for the female flies and for the total. The smallest chi-square, 11.24 for the young females, has a probability of chance occurrence of about 0.001. The probabilities of the deviations observed for the old males and in the total being due to chance are negligible. Table 5 shows that the observed and the expected frequencies for the young males agree well enough, but the heterozygotes are significantly more frequent than expected among young females as well as among old males. The total shows a highly significant excess of heterozygotes and a deficiency of homozygotes.

It must be noted that the excesses of heterozygotes and the deficiencies of homozygotes are very likely even greater than the data in tables 4 and 5 indicate. As stated above, the technique of distinguishing the homo- and heterozygotes was such that approximately one in every thirty-two heterozygotes must have been misclassified as a homozygote. The expected numbers of the different classes must, therefore, be corrected according to the following formulae:

ST/ST or AR/AR	$q^2+1/64[2q(1-q)]$
ST/CH or AR/CH	$62/64[2q(1-q)]$
CH/CH	$(1-q)^2+1/64[2q(1-q)]$

If these corrections are introduced, the observed and the expected values for the total in cage number 22 become as follows (compare with table 4):

	ST/ST	ST/CH	CH/CH
Observed	57	169	29
Expected	80.5	122.0	52.5
Difference	−23.5	+47.0	−23.5

For the total in cage number 23 the observed and the expected figures become as follows (compare with table 5):

	AR/AR	AR/CH	CH/CH
Observed	80	196	58
Expected	97.5	161.0	75.5
Difference	−17.5	+35.0	−17.5

It is, then, safe to conclude that, among the adult flies developed in the population cages numbers 22 and 23, heterozygotes are quite appreciably more frequent, and homozygotes less frequent, than demanded by the HARDY-WEINBERG formula. It should, however, be recalled that the HARDY-WEINBERG proportions were found to be relatively only slightly disturbed among the larvae coming from the eggs deposited in the population cages but grown under the near-optimal conditions in culture bottles (tables 2 and 3). It follows that a selective mortality takes place among the individuals homozygous for the gene arrangements ST, CH, and AR in the population cages. At just what stage of the life cycle the selective mortality occurs does not appear from the data, except that it must lie between the egg stage and the emergence of the adult insect from the pupa.

TABLE 5

Genetic structure of adult flies in population cage No. 23.

SEX AND AGE		ZYGOTIC FREQUENCIES			χ^2	GAMETIC FREQUENCIES IN PERCENT	
		AR/AR	AR/CH	CH/CH		AR	CH
Young ♀♀	Observed	24	63	13		55.5	44.5
	Expected	30.8	49.4	19.8			
	Difference	− 6.8	+13.6	− 6.8	7.57		
Old ♂♂	Observed	22	63	15		53.5	46.5
	Expected	28.6	49.8	21.6			
	Difference	− 6.6	+13.2	− 6.6	7.04		
Young ♂♂	Observed	34	70	30		51.5	48.5
	Expected	35.5	66.9	31.5			
	Difference	− 1.5	+ 3.1	− 1.5	0.27		
Total	Observed	80	196	58		53.3	46.7
	Expected	94.8	166.3	72.9			
	Difference	−14.8	+29.7	−14.9	10.66		

The finding that selective elimination of homozygotes takes place in the population cages is important because it proves that the hypothesis of natural selection is correctly applied to our data. An attempt may now be made to ascertain whether this selective mortality occurs on a scale sufficient to account for the speed of the changes in frequencies of the gene arrangements observed in cages numbers 22 and 23 (table 1), as well as in the experiments described earlier by WRIGHT and DOBZHANSKY (1946).

WRIGHT and DOBZHANSKY (1946) have published the results of four experiments (numbers 8, 11, 13, and 19) in which population cages with mixtures of ST and CH chromosomes were kept at temperatures close to 25°C. Two more experiments of this kind are described in the present article (tables 1 and 9). The results of all experiments are summarized in table 6. The column marked q_0 gives the frequencies of ST chromosomes found in the successive samples taken in each cage. The column Δq_0 shows the differences between the frequencies q_0 in the successive samples. The time intervals elapsed between the samples are indicated in weeks. Since a fly generation takes about 3.5 weeks in population cages at 25°C, the net differences, Δq_0, must be converted into differences per 3.5 week periods; the resulting figures are given in the column marked "Δq_0 per generation." The figures in the left part of the table are "Uncorrected" for the systematic error arising because a slight but perceptible differential mortality of ST/ST and CH/CH homozygotes takes place even when the larvae are raised on abundant food (see above). If this differential mortality is corrected for, the observed frequencies of ST chromosomes, q_0, give the real frequencies, q, and the "Crude Δq" and the "Δq per generation" can easily be computed (table 6).

The selective disadvantages of the homozygotes ST/ST and CH/CH, denoted s and t respectively, the adaptive values W, and the frequency of ST chromosomes, $\hat{q}$, at which the population of an experimental cage reaches equilibrium, have been computed from the data in table 6 by PROFESSOR SEWALL WRIGHT with the aid of the equations given in WRIGHT and DOBZHANSKY, 1946 (see particularly equation 11, on page 142). From the uncorrected data in table 6 the following results are obtained:

GENOTYPE	FREQUENCY	W		
ST/ST	q^2	0.766	s=0.234	
ST/CH	$2q(1-q)$	1.000		$\hat{q}$=0.724
CH/CH	$(1-q)^2$	0.387	t=0.613	

The corresponding values derived from the corrected data are:

GENOTYPE	FREQUENCY	W		
ST/ST	q^2	0.762	s=0.238	
ST/CH	$2q(1-q)$	1.000		$\hat{q}$=0.723
CH/CH	$(1-q)^2$	0.379	t=0.621	

The corrected and the uncorrected data yield substantially the same estimates. The adaptive value of ST homozygotes is, under the conditions of the experiments, approximately 76 percent of that of the ST/CH heterozygotes; the adaptive value of CH homozygotes is only 38 percent of that of the

TABLE 6

Summary of data on rate of change in the frequency of ST gene arrangement in population cages kept at temperatures close to 25°C. Further explanation in text.

EXPERIMENT NO.	INTERVAL (WEEKS)	UNCORRECTED			CORRECTED		
		q_0	CRUDE Δq_0	Δq_0 PER GENERATION	q	CRUDE Δq	Δq PER GENERATION
8	4.5	0.520	+0.083	+0.065	0.503	+0.091	+0.071
	3.0	0.603	+0.030	+0.035	0.594	+0.033	+0.038
	4.5	0.633	+0.060	+0.047	0.627	+0.065	+0.051
	—	0.693	—	—	0.692	—	—
11	5.5	0.463	+0.154	+0.098	0.440	+0.169	+0.107
	4.0	0.617	+0.046	+0.040	0.609	+0.050	+0.044
	4.5	0.633	−0.026	−0.020	0.659	−0.028	−0.022
	—	0.637	—	—	0.631	—	—
13	7.0	0.292	+0.255	+0.127	0.262	+0.270	+0.135
	3.5	0.547	+0.036	+0.036	0.532	+0.040	+0.040
	—	0.583	—	—	0.572	—	—
19	6.0	0.383	+0.147	+0.086	0.355	+0.159	+0.093
	4.5	0.530	+0.103	+0.080	0.514	+0.113	+0.088
	5.5	0.633	−0.030	−0.019	0.627	−0.033	−0.021
	4.5	0.603	+0.050	+0.039	0.594	+0.054	+0.043
	4.5	0.653	0.000	0.000	0.648	0.000	0.000
	6.0	0.653	+0.051	+0.030	0.648	+0.056	+0.032
	7.5	0.704	+0.016	+0.007	0.704	+0.017	+0.008
	—	0.720	—	—	0.721	—	—
22	4.5	0.203	+0.120	+0.093	0.175	+0.118	+0.092
	4.5	0.323	+0.104	+0.081	0.293	+0.109	+0.085
	4.5	0.427	+0.120	+0.093	0.402	+0.130	+0.102
	—	0.547	—	—	0.532	—	—
24	4.5	0.896	−0.029	−0.023	0.902	−0.029	−0.022
	4.5	0.867	−0.017	−0.013	0.873	−0.017	−0.013
	4.5	0.850	−0.050	−0.039	0.856	−0.051	−0.040
	—	0.800	—	—	0.805	—	—

ST/CH heterozygotes. These values are, however, somewhat higher than those arrived at by WRIGHT and DOBZHANSKY, 1946, on the basis of the older data (the old values are 0.7 for ST/ST and 0.3 fo CH/CH homozygotes). The equilibrium frequency of ST chromosomes is about 72 percent, instead of the old figure, 0.7, given by WRIGHT and DOBZHANSKY.

The selective disadvantages of the homozygotes (s and t) and the selective values (W) can, however, be estimated also quite independently from the above, using the deviations from the HARDY-WEINBERG proportions which are

observed among the adult flies in the population cages. Indeed, the HARDY-WEINBERG proportions are approximately, though incompletely, realized among the larvae coming from the eggs deposited in the population cages but grown in culture bottles with abundant food (see above). Among the adult flies much greater deviations from these proportions are apparent. Consequently, a differential elimination of homozygotes takes place between the egg and the adult stages.

The fundamental equations with the aid of which the adaptive values and the selective disadvantages can be calculated from the deviations from the HARDY-WEINBERG proportions among the adult flies have been generously supplied by PROFESSOR SEWALL WRIGHT. They are as follows:

$$ST/ST = nq^2\,(1-s)$$
$$ST/CH = 2nq\,(1-q)$$
$$CH/CH = n(1-q)^2\,(1-t)$$
$$\hat{q} = t/(s+t) = 0.7, \text{ from WRIGHT and DOBZHANSKY (1946)}$$

In these equations, q is the unknown frequency of ST chromosomes among the gametes from which the examined sample of the adult flies came (q_0 being the frequency after the selection has taken place, cf. table 6), s and t the selective disadvantages of ST/ST and CH/CH zygotes, n the unknown number of zygotes before selection, and $\hat{q}$ the equilibrium frequency of ST chromosomes in population cages containing mixtures of ST and CH. The data in table 4 can now be used to estimate the adaptive values, W. The observed numbers of the ST/ST, ST/CH, and CH/CH zygotes are corrected for the misclassified heterozygotes (see above). The adaptive values, W, for ST/ST and CH/CH zygotes equal $(1-s)$ and $(1-t)$ respectively. The results of the calculations made by PROFESSOR WRIGHT are shown in table 7.

TABLE 7

Estimates of the adaptive values of ST/ST, ST/CH, and CH/CH zygotes derived from the data in table 4.

ZYGOTE	YOUNG ♀♀				OLD ♂♂				AVERAGE W
	OBSERVED	CORRECTED	CALCULATED	W	OBSERVED	CORRECTED	CALCULATED	W	
ST/ST	31	29.7	41.6	0.714	26	24.6	37.0	0.665	0.690
ST/CH	83	85.6	85.6	1.000	86	88.8	88.8	1.000	1.000
CH/CH	16	14.7	44.0	0.334	13	11.6	53.3	0.218	0.277
	$n=171.2$	$q=0.493$	$q_0=0.558$		$n=179.1$	$q=0.455$	$q_0=0.552$		

The estimates of W derived from the data on females and on males are, as could be expected, somewhat different. Their averages give the most reliable values that can be secured. These values, 0.69 for ST/ST and 0.28 for CH/CH, agree remarkably well with the estimates, 0.7 and 0.3 respectively, arrived at by WRIGHT and DOBZHANSKY, 1946, from the data on changes in the frequen-

cies of ST and CH with time in the population cages. The agreement is somewhat less close with the improved estimates, 0.76 and 0.38 respectively, arrived at from the more complete data (see page 152). In any case, it is justified to conclude that the differential mortality (inferred from the data on the deviations from the HARDY-WEINBERG proportions among the adult flies in the population cages) is more than sufficient to account for the rate of change in the frequencies of ST and CH chromosomes observed with time in these population cages.

The adaptive values of the AR/AR, AR/CH, and CH/CH zygotes have been calculated by PROFESSOR WRIGHT from the data in table 5; the results are shown in table 8.

TABLE 8

Estimates of the adaptive values of AR/AR, AR/CH, and CH/CH zygotes derived from the data in table 5

ZYGOTE	YOUNG ♀♀				OLD ♂♂				YOUNG ♂♂				AVERAGE
	OBSERVED	CORRECTED	CALCULATED	W	OBSERVED	CORRECTED	CALCULATED	W	OBSERVED	CORRECTED	CALCULATED	W	W
AR/AR	24	23.0	31.7	0.726	22	21.0	28.6	0.734	34	32.9	36.0	0.914	0.804
AR/CH	63	65.0	65.0	1.000	63	65.0	65.0	1.000	70	72.2	72.2	1.000	1.000
CH/CH	13	12.0	33.3	0.360	15	14.0	36.9	0.379	30	28.9	36.2	0.799	0.542
	$n=130.0$	$q=0.494$	$q_0=0.555$		$n=130.5$	$q=0.468$	$q_0=0.535$		$n=144.4$	$q=0.499$	$q_0=0.533$		

The best estimates from the available information are adaptive values of about 0.80 for homozygous AR and about 0.55 for homozygous CH. The selection against the homozygotes appears, then, to be weaker in this case than it is for the ST and CH homozygotes in the population cages containing the latter two gene arrangements. Examination of table 1 suggests, however, that the rates of changes in the frequencies of ST and AR gene arrangements competing against CH are much the same in population cages numbers 22 and 23. If so, the adaptive values of AR/AR homozygotes and AR/CH heterozygotes should be about the same as those of ST/ST and ST/CH respectively. If this will be confirmed in future experiments, it would follow that the disturbances in the HARDY-WEINBERG equilibrium proportions shown in tables 5 and 8 are insufficient to account for the speed of the changes in the frequencies of AR gene arrangement observed with time in the population cages. If so, the explanation will have to be looked for in differences of adaptive values among the adults of different chromosomal constitution.

THE EQUILIBRIUM IN POPULATION CAGES

In all the experiments conducted at high temperatures reported by WRIGHT and DOBZHANSKY (1946) as well as in the present article, the frequencies of ST or AR chromosomes were observed to increase at the expense of CH chromosomes. In these experiments the initial populations of the cages contained either equal numbers of CH and ST or AR, or else CH chromosomes had

higher initial frequencies than their stronger competitors. However, CH chromosomes were never supplanted entirely by ST or AR; instead, whenever the population cages were kept for a sufficiently long time, an equilibrium was reached. For ST and CH, this equilibrium lies close to 70 percent ST and 30 percent CH. The existence of this equilibrium is due to the heterozygotes ST/CH exceeding in adaptive value both homozygotes. The equilibrium is reached when the average adaptive level in the population as a whole is at a maximum. An equilibrium is, however, a state which can be attained from two opposite directions. Provided that the interpretation of the above data is correct, it follows that if the initial population of a cage contains more than 70 percent ST and less than 30 percent CH, the frequency of CH must increase with time at the expense of ST. To test this, a mixture of 2235 flies containing 89.6 percent ST and 10.4 percent CH chromosomes was introduced in population cage number 24 on October 20, 1945. At least 19 "kinds" of ST and 12 "kinds" of CH chromosomes went into the mixture (cf. WRIGHT and DOBZHANSKY 1946). Samples of eggs were taken in cage number 24 in late November and late December 1945, and in late January 1946. The results obtained are reported in table 9.

TABLE 9

Percentage frequencies of ST and CH chromosomes in the population cage No. 24

TIME	ST	CH	n
Initial (October)	89.6	10.4	
November	86.7	13.3	300
December	85.0	15.0	300
January	80.0	20.0	300

In three months the frequency of CH has seemingly doubled—it increased from about 10 percent to about 20 percent. The change is just significant statistically (it is below the conventional level of significance if only the samples from November to January are taken into account). However, the observed rate of change is as fast as expected if the adaptive values of the chromosomal types ST/ST, ST/CH, and CH/CH are close to respectively 0.7, 1.0, and 0.3, as estimated by WRIGHT and DOBZHANSKY (1946). Indeed, starting with a population of 90 percent ST and 10 percent CH, one should theoretically get a population with 82.5 percent ST and 17.5 percent CH in about three generations. Unfortunately, cage number 24 contracted a mite infection in January of 1946 and could not be followed further.

The experiment just described is the first among the experiments thus far conducted with population cages in which the frequency of CH chromosomes has apparently increased at the expense of ST chromosomes. The equilibrium frequencies of the gene arrangements can be approached from both directions. An even more convincing demonstration of this has recently been obtained in experiments with population cages which contained flies descended from wild ancestors collected at Mather, California. These experiments will be reported

in detail later, and only a preliminary statement can be given here. Population cage number 29 was started on December 22, 1945, with about 70 percent ST and 30 percent AR chromosomes. In less than six months it reached an equilibrium at the level of about 55 percent ST and 45 percent AR. Population cage number 32, started on the same day as number 29, had initially about 20 percent ST and 80 percent AR. In September 1946 this cage contained 52 percent ST and 48 percent AR. It appears, then, that ST and AR chromosomes from the Mather locality tend, under the conditions of the popultaion cages, to reach an equilibrium at about 50 percent of each.

DISCUSSION

The deviations from the HARDY-WEINBERG ratios of homo- and heterozygotes observed among the adult flies in the population cages constitute a proof of differential survival among the chromosomal types involved. This proof of differential survival, and hence of natural selection, is independent of the demonstration of the action of selection by means of observations on the changes in frequencies of the gene arrangements with time in the population cages. That is, even if one did not know that the frequencies of these gene arrangements actually change under certain conditions (temperature, etc.) in the population cages, one could predict that such changes must occur.

What physiological properties of the carriers of the gene arrangements studied are responsible for the differential survival is unknown. In any case, the differential survival takes place in some environments and not in others. Relatively little differential elimination occurs when larvae are kept without crowding on abundant food (tables 2 and 3). WRIGHT and DOBZHANSKY (1946) have found no changes in the population cages kept at 16½°C, which means that at that temperature the adaptive values of the gene arrangements differ relatively little. Observations in nature show further that while in summer ST chromosomes displace CH, in spring CH chromosomes displace ST (DOBZHANSKY 1943). This suggests that under certain conditions, not yet duplicated in the experimental population cages, carriers of CH may even be superior to those of ST. The experiment conducted in the population cage number 24, in which CH increased in frequency at the expense of ST (see above), does not reproduce the spring conditions in which the natural populations on Mount San Jacinto live. Indeed, what happened in cage number 24 was that the equilibrium of ST and CH chromosomes, which lies at about 70 percent of the former and 30 percent of the latter, was approached by a population which had originally 90 percent ST and only 10 percent CH. All other experiments so far conducted had starting frequencies of ST below and of CH above the equilibrium values. The cyclic changes which occur in nature apparently entail changes in the equilibrium values themselves. In summer the equilibrium is in favor of ST, in autumn and winter ST and CH are equivalent, while in spring the equilibrium changes in favor of CH.

Drosophila pseudoobscura is not unique in having cyclic seasonal changes in the frequencies of genetic variants in natural populations. The excellent work

of DUBININ and TINIAKOV (1945, 1946a, b) has disclosed that a certain inversion, called II-1, increases in frequency in the populations of *D. funebris* in the city of Moscow from spring till early autumn. However, the frequencies of the same inversion dwindle during winters. DUBININ and TINIAKOV were able to show that carriers of this inversion survive artificially induced hibernation less frequently than do carriers of the alternative gene arrangement. This lessened survival ability affects inversion homozygotes as well as heterozygotes. The adaptive value of the inversion II-1 is, consequently, higher than that of its alternative gene arrangement during the warmer season, but lower during hibernation. Furthermore, this inversion is quite common in urban populations but relatively rare in rural districts, indicating that its adaptive value is higher in urban than in rural environments.

The fact that in *D. funebris* both homozygotes and heterozygotes for the inversion II-1 are discriminated against during hibernation makes the genetic mechanism discovered in this species by DUBININ and TINIAKOV less perfect than the one existing in *D. pseudoobscura*. An unusually severe or prolonged winter might throw the inversion out of the population altogether, notwithstanding the fact that the same inversion has a high adaptive value under summer conditions. In *D. pseudoobscura* the inversion heterozygotes are superior in adaptive value to the corresponding homozygotes. The superiority of heterozygotes permits natural populations to respond to spatial as well as to temporal variations in the environment without depletion of their store of genetic variability; such a depletion would lead to loss of evolutionary plasticity. Indeed, the chance of accidental loss of any one gene arrangement is reduced to a minimum because the inversion frequencies reach an equilibrium at which all gene arrangements are retained. Retention of plasticity is possible, however only at a sacrifice of immediate fitness, because the plasticity entails continuous production of relatively ill-adapted inversion homozygotes. The constitution CH/CH acts, as shown above, as a semilethal at temperatures above 20°C. This opposition of plasticity and fitness has been pointed out by HALDANE (1937) and the writer (DOBZHANSKY 1937, 1938), and emphasized especially by MATHER (1943). The situation found in *D. pseudoobscura* illustrates this opposition most vividly.

Looked at from another angle, the superiority of inversion heterozygotes over the homozygotes furnishes a mechanism for the maintenance of heterosis. In a species in which a wide outbreeding is the rule, a mutant gene, or a gene complex which is deleterious to homozygotes may nevertheless be favored by natural selection if it is adaptive in heterozygotes with other genes or gene complexes. If favorable effects are produced by a complex of linked genes which taken separately are less advantageous or even deleterious, an inversion which binds together this complex acquires a selective advantage. Selection consequently favors increasing the variety of inversions with different gene complexes, because the greater the variety of inversions present in a population the less frequently will the relatively ill-adapted homozygotes be produced. Natural populations of some species of Drosophila are composed mainly of inversion heterozygotes. This is in general true of *D. pseudoobscura*; however

such species as *D. virilis*, *D. repleta*, and *D. hydei* seem to be free of inversions, (PATTERSON, STONE and GRIFFEN 1940, WHARTON 1942, WARTERS 1944) and even in *D. pseudoobscura* populations of some territories consist of inversion homozygotes. CARSON and STALKER (1947) failed to detect seasonal changes of inversion frequencies in *D. robusta* near St. Louis, and the writer has reason to believe that no such seasonal changes occur in populations of *D. persimilis* in the Sierra Nevada of California. No seasonal changes have been found in the population of *D. pseudoobscura* at Keen Camp, on San Jacinto. Comparative studies on the population structure in different species living in different environments are a promising field for investigation.

SUMMARY

A mixture of several strains of *Drosophila pseudoobscura* with roughly 20 percent of Standard (ST) and 80 percent of Chiricahua (CH) chromosomes was introduced in a population cage. In about three months this cage contained approximately 55 percent ST and 45 percent CH. The initial population of another cage consisted of roughly 21 percent Arrowhead (AR) and 79 percent CH; three months later AR rose to 57 percent and CH fell to about 43 percent.

Samples of eggs deposited by the flies in the population cages were taken, and the larvae coming from these eggs were grown under optimal conditions. Chromosomal heterozygotes (ST/CH or AR/CH) and homozygotes (ST/ST, AR/AR, CH/CH) are found among such larvae in proportions close to those demanded by the HARDY-WEINBERG formula, although there is a slight but significant excess of heterozygotes. Consequently, the flies in the population cages are panmictic with respect to the chromosomal constitution, and there is relatively little differential mortality between the egg and the late larval stages under optimal conditions.

The HARDY-WEINBERG proportions are not realized among adult flies developed in the population cages. There is a very appreciable excess of heterozygotes and a deficiency of homozygotes. A selective elimination of a part of homozygotes through differential mortality must take place in the population cages; this differential mortality occurs probably in the larval stage.

The differential mortality of homozygotes in the cage which contained ST and CH was more than sufficient to account for the speed of the changes in the frequencies of these chromosomes observed in this cage. On the other hand, in the cage with AR and CH chromosomes the differential mortality indicated by the data may or may not be sufficient. Natural selection in the population cages need not, however, be based on differential mortality alone; differences in longevity, fecundity, etc. of adult flies may also exist.

The selective process taking place in population cages leads to establishment of an equilibrium at which all the gene arrangements present in the initial population of a cage are retained. For ST and CH chromosomes this equilibrium lies at about 70 percent ST and 30 percent CH. It is demonstrated that this equilibrium can be approached both when the initial populations have less and when they have more than 70 percent ST.

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THE EFFECT OF LOW TEMPERATURE UPON THE FREQUENCY OF X-RAY INDUCED MUTATIONS

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INVESTIGATIONS of the combined effect of temperature and X-rays on mutation rate and chromosomal aberrations have given conflicting results. The early work of STADLER (1928, 1930) on Hordeum and that of MULLER (1930) on Drosophila admitted the possibility of a temperature effect. Negative results were obtained by TIMOFÉEF-RESSOVSKY (1934) on mutation rate in Drosophila and by MAKHIJCINI (1944) on chromosomal aberrations. However, MEDVEDEV (1935) did find a higher mutation rate in flies irradiated at low temperatures, and higher frequencies of chromosomal aberrations were found by PAPALASHIVILI (1935), MICKEY (1939) and HERSKOWITZ (1946). In order to clear up any doubt on this fundamental question the author has reinvestigated the problem by using a different technique and a broad range of dosages.

The stocks selected for this investigation were (1) Canton-Special (*C.S.*) for the wild genotype because of its low spontaneous mutation rate and high X-ray sensitivity (DEMEREC 1937) and, (2) sc^8 *B In-S* w^a, known as *Muller-5* (*M-5*) for the crossover-suppressor. This inversion stock possesses certain advantages over the *ClB* used in all earlier lethal-induction investigations. Unlike *ClB*, it is homozygous-viable; sc^8 is a well-known inversion involving nearly the entire genetic map of chromosome 1; *In-S* is an inversion within sc^8. The combination of an inversion within an inversion reduces crossing over to practically nil, which is not true for the *ClB* stock. The major sc^8 inversion contains the dominant duplication mutant Bar eye (*B*) and the recessive mutant apricot eyecolor (w^a). Also, unlike *ClB*, all socalled nondisjunctional females can be eliminated with absolute certainty on the basis of the recessive apricot eyecolor.

The *C.S.* males for raying, both experimental and control, were removed from the stock cultures and isolated in culture bottles for three days prior to treatment, in order to build up a large mature sperm reservoir. For treatment these males were placed in small gelatin capsules. The experimentals were placed in a refrigeration unit and the controls into an identical unit but with no refrigeration.

These units consisted of paraffined cylindrical paper "ice cream" containers. A pint-size container with a paraffin floor was fixed firmly into the deep paraffin floor of an outer gallon-size container. Each container top was made into a cellophane window. The unit for the cold series was kept in a 0.5°C constant temperature vault and on the day for raying, the space between the containers was filled with crushed ice.

The capsules containing the *C.S.* males for the cold series were placed in the refrigerator unit 30 minutes prior to raying, in order to lower their temperature to 0.5°C. For raying, the units were placed, one at a time, under a standard COOLIDGE X-ray tube with the flies 20 cm distant from the target. The five dosages used were 600 r units and multiples up to 3600 r units, with the exception of 3000 r units. No filter was used.

Virgin *M-5* females were mated with X-rayed *C.S.* males. The F_1 *M-5/C.S.* females were "caged" with *M-5* males for several days, after which they were transferred to shell vials, one female to two males per vial. In F_2, if no lethal were induced, the males should be of two very distinct phenotypes, namely, *C.S.*, round red eyed, and *M-5*, Bar apricot. On the other hand, were a lethal induced, the males should be only Bar apricot.

The X-ray controls were irradiated at room temperature. Although the parents of both experimentals and controls were not discarded during the following ten-day period, only the heterozygous F_1 females of the first six days emergence were chosen for study.

The results are shown in table 1. The average frequency of lethal mutations for all dosages was found, on the average, to be 2.30 times greater for the low temperature series than for the room-temperature series. For each dosage alone this excess was as follows:

600r units—3.09×
1200r units—2.07×
1800r units—2.17×
2400r units—1.84×
3600r units—2.33×

Plotting the frequencies of lethal mutations against dosage for each of the two series two divergent straight-line curves result, lines on which the frequency of any dosage whatsoever under the same experimental temperature should with reasonable expectancy fall. All the mutation values recorded are in terms of total lethality and do not include borderline semi-lethals.

No lethal appeared in either the nonirradiated control series so low temperature alone under the conditions of the experiment did not induce lethal mutations.

Sterility in the cold temperature series gives added proof of the effect of low temperature. Cold treatment without irradiation seemed to improve viability, compared with the flies irradiated at room temperature. Referring to the table it will readily be seen that for cold treatment with irradiation a sharp drop in viability results with increase in dosage, whereas the viability fluctuations in the warm series give no such curve. Although a different type of curve, the variation for viability at the low temperature is equally as smooth as that for mutation frequency.

MULLER (1930) observed what seemed to be a temperature effect as expressed in lethal frequency of flies irradiated at about 2300 r units at a low temperature (8°C) and at a high temperature (34°C), 8.2 percent and 6.2 percent respectively. At that time he was inclined to interpret it as a secondary radiation effect but was not satisfied with that explanation. Had MULLER ir-

radiated his flies at or close to 0°C his results would have been much more convincing in reference to the effect of low temperature. However, his values are in accord with what might be expected for an intermediate temperature. Following the suggestion of MULLER, MEDVEDEV (1935) repeated the experiment and found that a definite difference in lethal mutation frequencies did exist between his cold series at 0°C and at room temperature. Using three

TABLE 1

Data on X-ray induced mutations at high and low temperatures.

DOSAGE	TEMPERATURE	AVERAGE *M5/+* PER CULTURE BOTTLE	% VIABILITY	CHROMOSOMES TESTED	LETHALS	% LETHALS
Control	25°	265	88.33	300	0	0
Control	0°	280	94.33	322	0	0
600	23°	201	67.00	118	1	0.85
600	0°	239	98.74	304	8	2.63
1200	27°	271	90.33	254	10	3.94
1200	0°	125	41.67	245	20	8.16
1800	23°	332	110.67	384	22	5.73
1800	0°	68	22.67	185	23	12.43
2400	26.8°	334	111.33	280	26	9.29
2400	0°	59	19.67	111	19	17.12
3600	28°	213	71.00	741	85	11.52
3600	0°	16	5.33	153	41	26.80

dosages 2500 r, 6000 r and 7000 r he obtained approximately twice as many lethal mutations at low as compared with room temperature; for low temperature 5.9 percent, 10.00 percent and 13.24 percent respectively as compared with room temperature 3.48 percent, 5.75 percent and 8.60 percent respectively. Independently, PAPALASCHWILI (1935) found that the combined action of X-rays and low temperature, 0° and lower, increased the frequency of translocations as compared with the action of X-rays alone. MICKEY (1939) likewise, following the implications contained in the investigations of PAPALASHWILI, found that, as with lethal mutations, low temperature combined with X-ray produced approximately twice as many translocations as X-ray at room temperature.

Since irradiation at low temperature produces an increase of both lethal mutations and chromosomal aberrations there is reason to believe that the two events may be related. Not only is low temperature more effective in producing X-ray induced mutations and chromosomal aberrations, but the dosage relations are linear in both cases. The recent work of LEA and CATCHESIDE (1945)

and of HERSKOWITZ (1946) shows that the two responses—lethal mutation and chromosome breakage—are related and that the great majority of X-ray induced lethals are probably produced at points of breakage. It is therefore to be expected that the frequency of both lethal mutations and chromosomal aberrations should respond in a similar manner to irradiation at low temperatures and show similar relations to X-ray dosage.

In response to criticisms relative to dosage effect of ice as opposed to air, tests indicate only 5.6 percent increase due to the use of ice in the apparatus.

SUMMARY

Special stocks of Drosophila were irradiated at various dosages from 600 to 3600 r at room temperature and at 0.5°C. For all dosages the frequency of induced lethal mutations were greater at the low temperature, with an average increment of ×2.3. The relation between dosage and frequency of lethal mutations was linear at both temperatures.

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CYTOGENETIC NOTES ON TOMATO SPECIES AND HYBRIDS[1]

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THE cultivated and the newly introduced wild species of tomatoes were studied in an exploratory way as to their fertility in cultivation, their crossability, and the appearance, sterility and cytology of some diploid and tetraploid interspecific hybrids. A later paper will describe segregation in their backcross and F_2 progeny.

SPECIES OF THE TOMATO GENUS

Since Lycopersicon was separated from the closely related Solanum, the species of tomatoes have been reduced by taxonomic revisions to six (MULLER 1940) or seven (LUCKWILL 1943), all probably indigenous to the West coast of South America or the adjacent Galapagos Islands. These species are naturally divided into two sections or subgenera:

I. EULYCOPERSICON, containing the two edible and widely cultivated species and their numerous natural and artificial hybrids and derived combinations. These are annual, mainly selfpollinating, species, generally lacking bracts and pseudostipules, and bearing glabrous, red (yellow, etc.) fruits containing the carotinoid pigments, lycopene and carotin. *L. esculentum* Mill. the common cultigen, has coarse, villose stems, while *L. pimpinellifolium* (Jusl.) Mill., the Red Currant tomato, has slenderer, smooth stems, raceme-like inflorescences, and smaller fruits.

II. ERIOPERSICON, including four or five, more or less woody-stemmed, perennial wild species, distributed along the narrow coastal strip or up mountain slopes from Ecuador to Chile, and in the Galapagos Islands. These species are probably crosspollinating (LUCKWILL 1943) and bear small, pubescent, clear-skinned, greenish-white fruits, often tinged, mottled or striped with purple, and containing chlorophyll and anthocyan pigments.

A. Species with simple inflorescence and leaf form, and lacking bracts and pseudostipules. *L. cheesmani* Riley, of the Galapagos, and *L. pissisi* Phil. (or *L. peruvianum var. humifusum* of MULLER 1940).

B. Species with forked inflorescences, more complex leaf forms, and with bracts and pseudostipules. *L. peruvianum* (L) Mill. is a coastal desert species with several varieties, including *dentatum* Dun (*L. chilense*), and probably *humifusum* C. H. Mull. (*L. pissisi*). *L. glandulosum*, C. H. Mull and *L. hir-*

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sutum, Humb. and Bonpl. are high Andean species with bitter fruits, containing solanin; the latter is of very rank growth, with stiff, coarse very villose and bright green stems; its variety *glabratum* is scantily villose.

FERTILITY OF SPECIES UNDER CONDITION OF CULTIVATION IN SOUTHERN ONTARIO

Varieties and strains of *L. esculentum* and *L. pimpinellifolium* generally bloom and fruit abundantly both outdoors and in; fruit-set in some is reduced in midwinter in the greenhouse. *L. peruvianum* and its many varieties, including *L. p. humifusum* (*L. pissisi*), which naturally range southward into the Temperate zone to latitude 30°, bloom profusely with us in the late summer and through the winter; though some stocks were practically non-fruiting the first year of their introduction, it proved easy to select strains which set regularly in the short days from September on. The two subtropical species, *L. glandulosum* and the typical *L. hirsutum* are similar in blooming in the field from late August until frost, and at irregular intervals during winters in the greenhouse: neither has fruited under our field conditions but *L. glandulosum* once in the three winters set 15 half sized fruits, one with fully formed seeds that failed to germinate. Their sterility here must involve chiefly female parts, since the pollen is good on inspection and in crossing and backcrossing tests. *L. hirsutum var. glabratum* is exceptional for that species in setting many seed-bearing fruits in the field.

The unfruitfulness of the more tropical, greenfruited species does not appear to be caused by sterility factors, ploidy, major chromosomal aberrations, or oppositional factors. In their native habitats collectors report them reproducing normally, and with us interplant pollinations are no more successfuls than selfing. The evidence clearly points to edaphic or climatic causes for their sterility when moved across the equator some 4000 miles to a new north temperate lowland environment (HOLCH *et al.*, 1941). In South America *L. hirsutum* and *L. glandulosum* "are obligate inhabitants of high altitudes, occurring usually above 7000 feet, and only rarely as low as 3500 feet" (MULLER 1940). Especially significant are the light relations, for the greenfruited species "are all short-day plants," flowering seldom in an 18-hour day, profusely in a 12-hour day, and setting some fruits in an 8-hour day (LUCKWILL 1943; ROBERTS and STUCHMEYER 1938).

In general the red-fruited species are more adaptable in their responses, and might be called euryphotoperiodic (tolerating a wide range of day lengths), while the greenfruited species, especially the tropical ones, are more narrowly specialized, or stenophotoperiodic, requiring short days for successful reproduction.

COMPATIBILITY OF TOMATO SPECIES

Persistent attempts were made by more than 1600 cross pollinations to intercross most of the available species (which did not include *L. cheesmani*) and varieties. (1) Within each of the polymorphic species (*L. esculentum* and *L. peruvianum*) it proved easy to obtain fertile hybrids between the varieties

however remote in geographic origin. Since *L. peruvianum* var. *dentatum* and var. *humifusum* both crossed readily with the species type, we are inclined to regard *humifusum* as a variety, rather than a distinct species (*L. pissisi*). (2) Intra-subgeneric interspecific crosses are also so easily made and so perfectly fertile between *L. esculentum* and *L. pimpinellifolium*, that these two species would be considered hardly more than well marked subspecies, if only genetic criteria of the validity of taxonomic categories were relied upon. From *L. peruvianum* ♀ × *L. glandulosum* ♂ viable seeds and vigorous hybrids were regularly obtained. (3) The successful inter-subgeneric crosses so far reported between the red and the greenfruited species include: (a) *L. esculentum* ♀ or *L. pimpinellifolium* ♀ with *L. hirsutum* ♂ (many cases by the authors), and between *L. esculentum* ♀ and *L. hirsutum* ♂ by others (PORTE *et al.* 1939, ALEXANDER *et al.* 1942), and (b) *L. esculentum* ♀ with *L. peruvianum* var. *dentatum* (*L. chilense*) ♂ (in rare instances only; HOLMES 1939, LESLEY and LESLEY 1943, ALEXANDER *et al.* 1942). On either red-fruited species *hirsutum* pollen produces quite regularly from one to 30 or more large viable seeds per fruit, which develop vigorous F_1 plants. It is still premature to claim this as an example of "one way compatibility" since *L. hirsutum*, like *L. glandulosum*, was female-sterile even with its own pollen under the ecological conditions obtaining.

We have had no success with *L. peruvianum* or *L. glandulosum* pollen on the Eulycopersicon species, though both pollens induce the development of nearly full-sized, seedless, parthenocarpic *esculentum* (LESLEY 1935) and *pimpinellifolium* fruits. A similar fruit development often occurs when *glandulosum* pollen is used on *peruvianum* or *hirsutum*, and *hirsutum* pollen on *glandulosum*. A nonspecific growth-promoting agent may occur widely in the genus.

Numerous artificial techniques to break the incompatibility barriers between other species also failed to produce viable hybrid seeds: pollination with a mixture of own and foreign pollen; shortening the style before pollination; and applying a sucrose solution to normal or cut styles. That the foreign pollen germinated on the stigmas of the species used as a female was determined by fixing and staining the styles (CUMMINGS *et al.* 1936). Embryo culture (LAIBACH 1925, SKIRM 1942, COOPER and BRINK 1940, and THOMPSON 1940) of collapsed abortive seed did not prove successful.

DESCRIPTIONS OF INTERSPECIFIC HYBRIDS

Our successful hybridization of *esculentum* with *hirsutum*, of *pimpinellifolium* with *hirsutum*, and of *peruvianum* with *glandolusum* has been mentioned.

The *esculentum-hirsutum* hybrids were emphasized because, practically, they might introduce valuable wild genes into the tomato, and theoretically, allelic and linkage relations may be studied through the many known *esculentum* chromosomes marker genes and linked groups. The *esculentum* mutant genes involved belonged to ten (of the expected twelve) groups (MACARTHUR 1934, BUTLER 1936) as follows: I. mottled leaves (*m*), dwarf

habit (d_1), peach fruit (p), and wooly or angora stem, leaves and fruit (Wo); II. yellow fruit flesh (r); III. brachytic habit (br), and colorless fruit epidermis (y); IV. potato leaf (c); V. fasciated fruit (f), green stem (a_1), leafy inflorescence (lf), and jointless pedicel (j); VI. lutescent foliage (l); VII. uniform color of unripe fruit (u_1), hairless stem (H) (from *L. pimpinellifolium*), and tangerine flesh color (t); VIII. stem becoming green (a_2); IX. very short internodes (d_2); and X. wilty foliage (wt). The locus of broad leaf (e) is not known.

Hybrids were made from repeatedly selfed tomato varieties, many homozygous also for mutant genes: X4071 from *Scarlet Dawn*, X4073 from *Tangerine*, X4075 from *Best of All*, X4077 from mutant *194*, X4078 from *3710*, X4250 from *902*, X4251 from *3213*, X4350 from *etu*, and X4353 from *Wooly*. Crosses X4351 from *Wooly* and X4352 from *902* were pollinated by *L. hirsutum* var. *glabratum*. Of these varieties *Scarlet Dawn* and *Best of All* show no mutant characters. *194* is an X-ray mutant with fine foliage and weak restricted growth. Tangerine has genes t and u; *3710* has m, d, p, r, and y; *902* has d, r, y, c, a, l; and *3213* has d_1, br, f, lf, j, H, a_2, d_2 and wt. *Wooly* is heterozygous for the dominant gene Wo, since $WoWo$ is lethal.

The 16 distinctive recessive mutant characters from the *esculentum* parent (dwarf, potato leaf, green stem, etc.) are in general completely recessive also in the *esculentum-hirsutum* hybrids. This is shown for dwarf and potato leaf in fig. 1, A, and for dwarf and wilty leaf in fig. 1, B. *Hirsutum* apparently supplies a homologous and fully dominant normal allele for each of these recessive genes representing ten chromosomes out of the set of 12. This interpretation is confirmed by the clear-cut segregation of the recessives in a roughly 1:1 ratio from the backcross of *esculentum* ♀ × F_1 ♂, while the progeny from F_1 ♀ × *hirsutum* ♂ are hardly distinguishable from pure *hirsutum*. These recessive characters have already been recovered in various combinations in selections from the first backcross.

While the F_1 hybrids are strongly *hirsutum*-like with respect to the recessive mutant differences, they tend to be more nearly intermediate between the parents in most of the normal specific and subgeneric contrasts. Dominance here is often partial or lacking. Such "blending" of characters is evident (table 1, left) in various degrees in general habit of growth, stem size and hairiness, development of pseudostipules and floral bracts, the shape, size and number of leaflets (fig. 1, A–C), flower size and shape (fig. 2, A–C), and seed size, flatness and pubescence. The F_1 fruits are oblate, 2–3 loculed, definitely smaller than intermediate, moderately or faintly striped with purplish, less leathery-skinned than *hirsutum*, and have tastes reminiscent of both species. The dried foliage has the "celery salt" odor of *hirsutum*.

The fruit epicarp and pericarp colors, the outstanding subgeneric key characters for the taxonomist, are especially interesting. The F_1 fruit epidermis is pale yellow when that of *esculentum* parent is deep yellow (YY), *hirsutum* being colorless; the hybrid is clearskinned when the *esculentum* parent is recessive clearskinned (yy). The hybrid fruit flesh is also nearly intermediate in degree of development of the two pigments from the red-

fruited *esculentum* (lycopene and carotin) and the two from the greenfruited *hirsutum* (chlorophyll and anthocyanin) (table 1) (LESLEY and LESLEY 1943). When the *esculentum* parent is tangerine fleshed (*tt*) the F_1 is greenish yellow; when it is yellow (*rr*) the hybrid is a pale yellow or whitish with green around the seeds and in the juice.

In the *pimpinellifolium-hirsutum* hybrids (X4070) the dominance of the *hirsutum* characters is even less pronounced, and the general habit and leaf

TABLE 1

Species and subgeneric characters in parents and hybrids.

	L. esculentum	*Esculentum-hirsutum hybrids*	*L. hirsutum*	*Pimpinellifolium-hirsutum hybrids*	*L. pimpinellifolium*
Bracts and stipules	Absent	Present, but not at every possible location	Present	Present often; moderately developed	Absent usually
Stem hairiness	Moderately hairy	Intermediate	Very hirsute	Intermediate	No long trichomes
Leaf margins	Entire to lobed	*Hirsutum*-like	Margins many-toothed Teeth entire or dentate	*Hirsutum*-like	Subentire or slightly undulate
Fruit hairiness	Glabrous	Sparsely hirsute to glabrous when ripe	Very hirsute	Approaching glabrous when ripe	Glabrous
Fruit markings	None	Purple midlocular stripe usually developed	Purple midlocular stripe	Purplish midlocular stripe	None
Fruit size (diameter)	6–10 cm	About 2.5 cm	About 2 cm	About 1.2 cm	About 1.0 cm
Fruit flesh colors	Red	Ochraceous Orange	Greenish-white	Ochraceous Orange	Red
	Tangerine Yellow	Primuline Yellow Naples Yellow			

shape (fig. 1, C, and table 1, right) are more like the Red Currant. Flower size and shape (fig. 2, D) are intermediate.

The same trends are shown also in the *peruvianum-glandulosum* cross, and in the *esculentum-peruvianum* var. *dentatum* hybrids (HOLMES 1939, LESLEY and LESLEY 1943).

In its crosses with *esculentum*, *pimpinellifolium* carries dominant alleles to all the recessive mutant genes and is partially dominant with respect to the key difference (villose, *hh* vs. smooth, *HH*, stem) and numerous other normal species contrasts.

HYBRID STERILITY

Many *esculentum-hirsutum* and *pimpinellifolium-hirsutum* hybrids were reared in pots and soil beds in the greenhouse through all seasons for three years, and in the field each summer. The natural set of fruits was low both on

TABLE 2

Natural yield of fruits and F_2 seeds from F_1 interspecific hybrids.

CROSSES	IN GREENHOUSE		IN FIELD		PERCENT GERMINATION
	FRUITS	SEEDS	FRUITS	SEEDS	
1. *Pimpinellifolium-hirsutum*					
X4070	4	39	13	75	90
2. *Esculentum-hirsutum*					
X4071	8	13	10	122	53
X4073			5	80	60
X4075			53	340	
X4077	37	9	7	72	50
X4078	15	157	18	43	54
X4250	0	0	0	0	
X4251	8	0	30	38	
3. *Peruvianum-glandulosum*					
X4257	15	ca. 400			

the plants isolated indoors, and on those left to uncontrolled pollination outside (table 2). The average number of large seeds per fruit varied from none to about twelve in the different crosses. Making the diploid hybrids tetraploid (figs. 9–10) did not restore, or even raise, their fertility.

Backcrosses were chiefly relied upon for genetic analysis beyond the F_1 generation. Of the four possible backcrosses, only those succeeded where the

TABLE 3

Results of artificial backcrossing of F_1 interspecific hybrids.

BACKCROSSES	POLLINATIONS	FRUIT SET	SEEDS	PERCENT GERMINATION
1. *Pimpinellifolium-hirsutum*				
X4070×*hirsutum*	27	9	5	
Red Currant×X4070	702	152	ca. 900	50
2. *Esculentum-hirsutum*				
X4071×*hirsutum*	43	7	128	
Scarlet Dawn×X4071	258	113	1730	73
X4073×*hirsutum*	13	5	54	
Tangerine×X4073	322	10	420	71
X4075×*hirsutum*	69	16	167	53
X4077×*hirsutum*	29	7	425	
194 mut.×X4077	58	3	170	
X4078×*hirsutum*	10	10	141	
3710×X4078	368	25	860	30
902×X4250	308	48	1380	70
3213×X4251	279	21	300+	31
3. *Peruvianum-glandulosum*				
peruvianum×X4257	3	3	104	

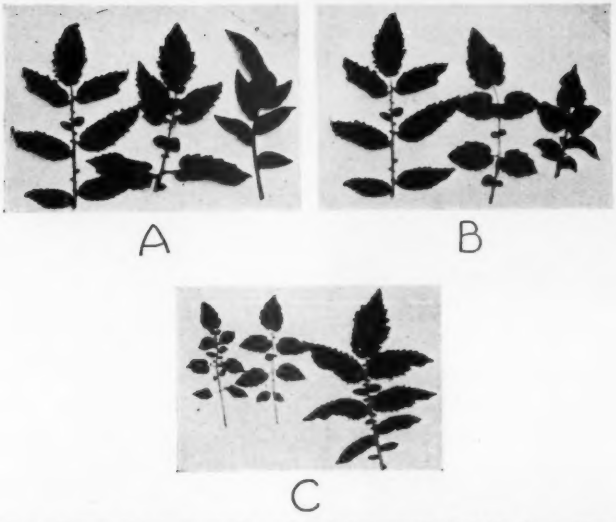


FIGURE 1.—Leaves of tomato species and their interspecific hybrids. A, *L. hirsutum*, left, X4250, center, and *L. esculentum* strain 902, right; B, *hirsutum*, left, X4251, center, and *esculentum* 3213, right; C, *L. pimpinellifoluim*, Red Currant, left, X4070, center, and *L. hirsutum*, right $\times\frac{1}{2}$.

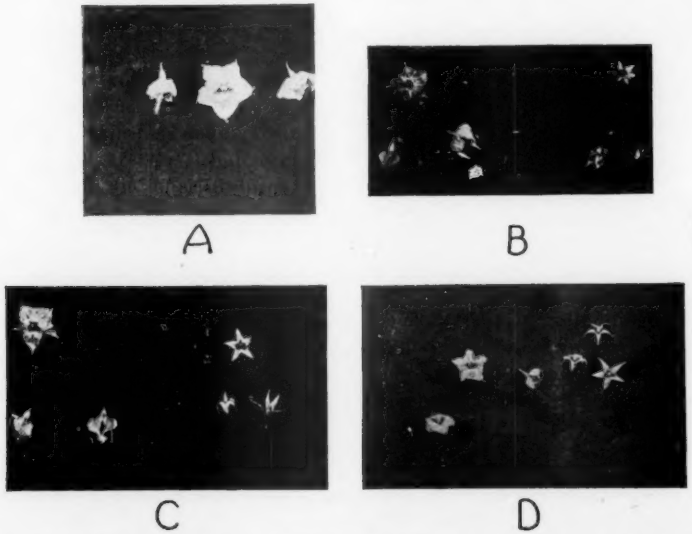
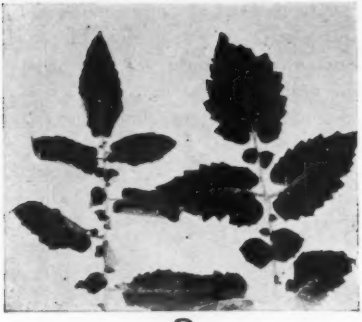


FIGURE 2.—Flowers of tomato species and their interspecific hybrids. A, *L. hirsutum;* B, left, X4251, and right, *esculentum* strain 3213; C, left, X4250, and right, *esculentum* strain 902; D, left, X4070, and right, *L. pimpinellifolium*, Red Currant. $\times\frac{3}{4}$.



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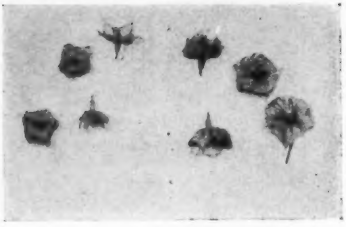

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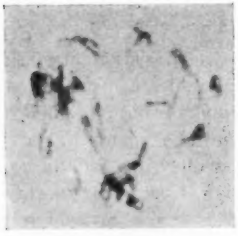
FIGURE 8, leaves, and FIGURE 9, flowers, of the *esculentum-hirsutum* F_1 hybrid, X4077, diploid at left, its tetraploid at right. $\times\frac{2}{3}$



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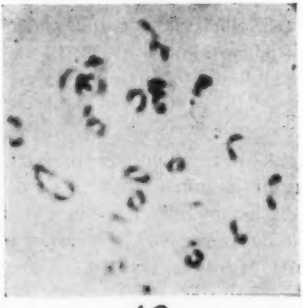

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FIGURE 10, X4077 at diplotene.
FIGURES 11, 12, and 13, X4075 at diakinesis, showing quadrivalents, trivalents, etc. $\times 1575$.
Chromosomes of tetraploid *esculentum-hirsutum* F_1 hybrids.

F_1 hybrids were used as males with *esculentum* or *pimpinellifolium*, or as females with *hirsutum*. From both types of backcrosses the fruit set rather readily, often with many good seeds (table 3).

Germination (tables 2–3) ranged from 31–73 percent in the backcrosses and from 50–90 percent in the F_2 samples tested, as compared with over 90 percent in the *esculentum* and Red Currant strains. Collapsed seeds often greatly outnumber the filled ones, and a selective mortality may well affect segregation ratios in the F_2 and backcross progenies. Many of the F_2 plants were completely sterile, and plant fertility from the backcrosses ranged from nil to that of the *esculentum* parent.

The *peruvianum-glandulosum* hybrid, X4257, had good, if not full, fertility both in the F_1 and in a backcross (tables 2 and 3). *Esculentum-pimpinellifolium* hybrids are as fertile as the parents.

CYTOLOGY OF TOMATO SPECIES AND HYBRIDS

The junior author began a cytological study to help understand the different compatibilities of the species, to determine the rôle of chromosome number and structure in causing sterility, and to interpret the more or less irregular types of segregation occurring in backcross and F_2 generations. Pollen mother-cells in meiosis were prepared by the anther squash smear technique (HILLARY 1940), being fixed in CARNOY'S aceticalcohol and stained with leucobasic fuchsin by the FEULGEN reaction.

All the known Lycopersicon species are diploids with 12 pairs of chromosomes. The complements differ slightly from species to species in a few recognizably larger chromosomes bearing distinctive knobs or trabants or having arms of different lengths (LESLEY 1935, LUCKWILL 1943). Cytogenetic studies of some related Solanum species are described by LAMM (1945).

PURE SPECIES. Meiosis in pollen formation was compared in *L. esculentum*, *pimpinellifolium*, *peruvianum* and its two main varieties, *glandulosum*, and in *hirsutum* and its glabrate variety. The process is strikingly similar in all these species to that described in *esculentum* (AFIFY 1933, UPCOTT 1935), *pimpinellifolium* (HUMPHREY 1932), and *peruvianum* (LESLEY and LESLEY 1943). At diplotene chiasmata are both interstitial and subterminal; they mostly terminalize. Twelve bivalents and no univalents were found at diakinesis. Chiasma frequency is slightly lower in *hirsutum* than in *esculentum* (table 4). The bivalents arrange normally on the equatorial plate at metaphase and anaphase I occurs without bridge formation or lagging chromosomes, except in one case in *L. peruvianum*.

DIPLOID F_1 HYBRIDS. Meiosis in the diploid interspecific hybrids, *esculentum-hirsutum* (figs. 3–5), *pimpinellifolium-hirsutum* (fig. 6), and *peruvianum-glandulosum* (fig. 7) differed little from that in the pure parent species. The pairing of chromosomes was regular, and 12 bivalents were invariably present as though no structural differences between chromosomes of parent species affected association. Even in the widest cross (*esculentum-hirsutum*, e.g. X4075) there were 13–15 chiasmata per cell, an average of 1.16 per chro-

mosome pair, as compared with 1.28 for *hirsutum* and 1.66 for *esculentum* (table 4). This cytological behavior is similar to that described in hybrids between more closely related species (*esculentum-pimpinellifolium*, AFIFY 1933), and between *esculentum* and *peruvianum* var. *dentatum* (LESLEY and LESLEY 1943). The data collectively are taken to indicate a close homology of chromosomes among species throughout the genus. Minor or cryptic structural differences between the sets might pass undetected, however, and

TABLE 4

Chiasma frequencies at diakinesis in 2n L. hirsutum, 2n and 4n esculentum (UPCOTT 1935),* *and in their 2n and 4n hybrids.*

MATERIAL	CHIASMATA PER 50 CELLS	CHIASMATA PER CELL	CHIASMATA PER CHROMOSOME PAIR
Diploids			
L. hirsutum	767	15.3±.14	1.28±.02
*L. esculentum**			1.66
F_1 hybrid	691	13.8±.11	1.16±.01
Tetraploids			
*L. esculentum**	1882	37.6	1.56
F_1 hybrid	1557	31.1±.4	1.30±.01

* (UPCOTT'S results were also based on counts made from 50 cells).

it is possibly significant that chiasma frequency is lower in the *esculentum-hirsutum* hybrid than in the parent species, in view of the similar relationship shown in Fritillaria and Triticum (DARLINGTON 1930, 1931).

TETRAPLOID F_1 HYBRIDS. Several *esculentum-hirsutum* F_1 hybrids were made tetraploid to determine whether fertility would be increased and chromosome pairing affected by doubling the chromosome number. Colchicine in lanolin, morpholine and stearic acid (DERMAN 1940) was applied to growing points. Not all responded alike to this treatment. Shoots selected as tetraploid on the basis of their general appearance, leaf thickness, size of stomata, flowers or pollen grains, were not always confirmed as tetraploids by cytological examination. In the proven tetraploids from X4075, X4077 and X4078 (figures 8–9), meiosis was very irregular and fertility was further decreased. At diakinesis the chromosomes formed bivalents, quadrivalents, trivalents and univalents (table 5, and figures 10–14), resembling those of autotetraploids. Tetraploid *hirsutum* was not available; both *4n* and *2n esculentum* have been adequately studied by UPCOTT (1935) and many others. The number of chiasmata are compared for the parent species, and their *2n* and *4n* hybrids (table 4). The data suggest a barely perceptible inclination toward preferential pairing, since quadrivalents are noticeably fewer and bivalents and trivalents more numerous in the *4n* hybrid than were reported in *L. esculentum*.

Lagging chromosomes were frequently observed in anaphase I and II of the tetraploid hybrid (figs. 15–16). The multivalent associations lead to unbalanced chromosome numbers in many gametes. The reduced number found in ten tetrads varied from 23 to 26, corresponding to 22 to 26 reported in

the tetraploid *esculentum* (UPCOTT 1935). This results, of course, in lowered pollen fertility and less formation of fruits and seeds.

These findings would be expected if the chromosomes of the parent species were sufficiently homologous to pair freely and thereby reduce any tendency

FIGURES 3, 4, and 5.—Diakinesis, anaphase I, and anaphase II, respectively, in pollen mother cells of the diploid *esculentum-hirsutum* F_1 hybrid X4075. ×1400.

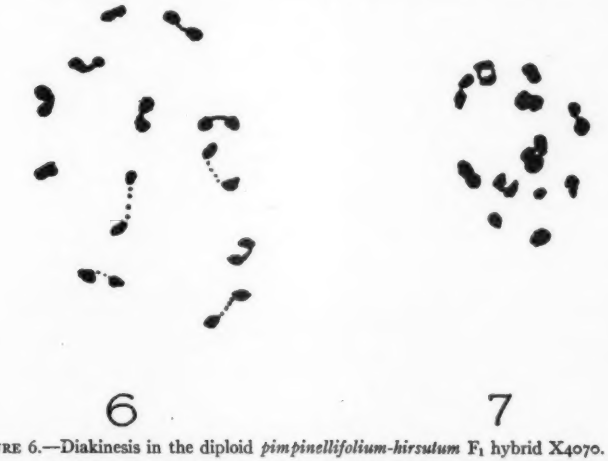

FIGURE 6.—Diakinesis in the diploid *pimpinellifolium-hirsutum* F_1 hybrid X4070.

FIGURE 7. Diakinesis in the diploid *peruvianum* typ.-*glandulosum* F_1 hybrid X4257. ×2100.

for preferential autosyndesis in the tetraploid pollen mother cells. Since the LESLEYS (1943) found from three to nine quadrivalents also in their *esculentum-peruvianum* hybrid, an "unexpected genetic similarity" of the chromosomes and genomes is indicated for the four species: *esculentum, pimpinellifolium, hirsutum,* and *peruvianum.*

DISCUSSION

The Lycopersicon genus consists of two subgenera or sections, and MULLER (1940) considers it possibly advisable ultimately to form a third by separating

off the two high-Andean species, *glandulosum* and *hirsutum*, from the other greenfruited species. Vigorous hybrids have now been obtained between each pair of these three most distinct sections of the genus. These three intersectional combinations, neglecting reciprocals, are represented by (1) *esculentum-peruvianum*, (2) *esculentum-hirsutum*, and (3) *peruvianum-glandulosum* crosses. Thus there are no likely serious hindrances to transferring and incorporating desired genes from any of the wild South American species into the

TABLE 5

Chromosome pairing at diakinesis in 50 pollen mother cells from the F_1 tetraploid esculentum-hirsutum (X4075), compared with that in autotetraploid esculentum (UPCOTT *1935*).

	UNIVALENTS	BIVALENTS	TRIVALENTS	QUADRIVALENTS
Frequency per 50 P.M.C.	39	991	45	61
Mean per cell	0.78	19.82	0.90	1.22
Mean number of chromosomes involved	0.78	39.64	2.70	4.88
Mean per cell in autotetraploid *esculentum* (UPCOTT)	0.56	15.80	0.08	3.86

commercial tomato, though bridging crosses, or embryo culture methods, etc. may be necessary in some cases. The causes of collapsed or abortive seeds in this group deserve further investigation.

Incompatibility appears to be most marked between the red-fruited species and *glandulosum* or *peruvianum*, and between *peruvianum* and *hirsutum*, but until cultural conditions are made suitable for full female and male fertility in the introduced species, it is too early to draw conclusions about crossability or to hold that certain crosses can be made one way and not in the reciprocal.

There is at least a rough correspondence between crossability, hybrid fertility and taxonomic relationship. If this rule were trusted implicitly it might suggest including *pimpinellifolium* as a subspecies of *esculentum*, and *pissisi* as a subspecies of *peruvianum*.

There is no evidence that different chromosome numbers, ploidy or major structural rearrangements in chromosomes are barriers to crossing or causes of the partial sterility of the diploid interspecific hybrids in Lycopersicon. In pollen formation at least, the chromosomes of hybrids behave much the same as in pure species. The relative sterility of the tetraploids from the hybrids further confirms this view. That endosperm or embryo abortion may occur in Lycopersicon seeds is suggested by the fact that only two of the four possible backcrosses are known to be successful, those using the F_1 ♀ with *hirsutum* and the F_1 ♂ with *esculentum*. For the present, genic, perhaps multiple factor, differences between the species seem the most likely causes of such partial hybrid sterility as exists.

The regular pairing of chromosomes in F_1 diploids, and the frequent multi-

valent associations and marked sterility of the F_1 tetraploids suggest a surprising likeness of the chromosomes even in the species which are phenotypically most dissimilar. The interspecific hybrids made tetraploid have the characteristic defects of autotetraploids, or of amphidiploids between species

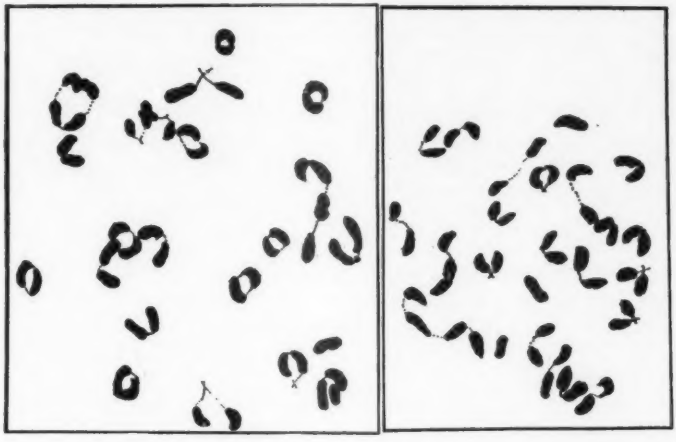

FIGURE 14.—Drawings of diakinesis in P.M.C. of the tetraploid *esculentum-hirsutum* F_1 hybrid, X4075. ×2470.

15 16

FIGURES 15 and 16.—Lagging chromosomes at anaphase I and II respectively in the tetraploid *esculentum-hirsutum* F_1 hybrid X4077. ×2100.

whose chromosomes are in general homologous. The sterility would be classed as haplontic rather than diplontic; genic rather than chromosomal. If structural hybridity of some degree exists, as suggested by a possibly reduced quadrivalent formation, it would be of the cryptic type due to minute chromosome rearrangements.

Evolutionary divergence and establishment of isolating mechanisms would appear to depend mainly on gene and modifier mutations and at most minor

reorganizations in chromosomes, which continue to carry homologous but not identical sets of factors.

Concerning the evolution of dominance, the outstanding relevant fact is that normal *hirsutum* alleles are strongly and immediately dominant over even the newest and rarest of the recessive *esculentum* genes. Dwarfness, lack of anthocyan pigment, broad or potato leaf, etc., are as recessive in the interspecific as in intraspecific crosses. The dominance of *hirsutum* alleles thus appears more like a primary quality than a secondary one gradually built up by selection of modifiers, as in FISHER'S (1931) hypothesis. The *esculentum-hirsutum* heterozygotes are more intermediate in ever-present normal species differences than in the seldom-recurring mutant differences. In some features the situation in the tomato species parallels that in cottons (SILOW 1939; HARLAND and ATTECK 1941).

SUMMARY

Two wild species of tomatoes, *Lycopersicon hirsutum* and *L. glandulosum*, introduced from the high Andes just South of the equator, bloom freely in short days but set no fruit in southern Ontario. Strains of *L. peruvianum* and its systematic varieties (including var. *humifusum*, or *L. pissisi*) and *L. hirsutum* var. *glabratum* are now fully fertile.

Interspecific crosses were attempted in over 1600 cross pollinations, and vigorous hybrids differing in fertility have now been obtained between species in each pair from the three most distinct sections of the genus. These include *peruvianum* ♀ with *glandulosum* ♂, *esculentum* (and *pimpinellifolium*) with *hirsutum*, and *esculentum* with *peruvianum* var. *dentatum*.

Many crosses for genetic analysis and practical uses introduced recessive marker genes from *esculentum*. These genes are also recessive in the *esculentum-hirsutum* hybrids, and segregate clearly in backcrosses. In species and subgeneric contrasts dominance tends to be partial or lacking.

An unexpected similarity between chromosomes and genomes of the species crossed is indicated by regular pairing in F_1 diploids, and by much multivalent association and marked sterility in F_1s made tetraploid by colchicine.

Evolutionary divergence and establishment of isolating mechanisms have evidently occurred in this group principally by gene and modifier mutation, and without benefit of ploidy, or major structural rearrangements.

ACKNOWLEDGMENT

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A SECOND ANALYSIS OF ANTIGENIC DIFFERENCES BETWEEN SPECIES IN COLUMBIDAE[1]

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EVIDENCE has been presented in previous papers that the antigenic characters of the red blood cells, by which one species differs from another, may segregate in the offspring of backcrosses of a species hybrid to one or the other of the parental species. Thus the antigenic complex by which *Columba guinea* differs from *C. livia* is made up of at least five or six distinct parts (IRWIN, *et al.* 1936). Similarly, a minimum of nine or ten such substances differentiate Pearlneck (*Streptopelia chinensis*) from Ring dove (*St. risoria*), and *vice versa* (IRWIN 1939). Further, Pearlneck is distinguished from Senegal (*St. senegalensis*) primarily by three of the nine or more cellular antigens peculiar to Pearlneck in comparison with Ring dove (IRWIN and COLE 1940).

Species hybrids have been obtained between Senegal and Ring dove, and backcross hybrids as well by mating the species hybrids to Ring dove with an accompanying segregation of cellular substances specific to Senegal in the hybrids of the first backcross generation (IRWIN and COLE 1945b). Matings to Ring dove of selected backcross hybrids have produced further segregation of these cellular components, and several have been obtained in unit-form. Essentially then, this report parallels for the specific antigens of Senegal the paper which described the isolation of the unit-antigens of Pearlneck. An abstract of the present paper has already appeared (IRWIN and CUMLEY 1946).

MATERIALS AND METHODS

Essentially the same immunological technics have been followed that have been described in previous papers (IRWIN and COLE 1936; IRWIN 1939). The system of matings to produce the species hybrids and the first backcross hybrid generation has also been given (IRWIN and COLE 1945a, b).

Except for the production of viable hybrids in the species crosses and backcrosses, which, at best, is an exceedingly slow procedure, one of the principal difficulties in these experiments has been to obtain a satisfactory antiserum, that is, one that contains antibodies to each of the different cellular antigens. Although between 100 and 150 rabbits have been immunized with Senegal

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cells during the past ten years, only one antiserum has been produced which contained antibodies against all the known cellular antigens with which a Senegal antiserum should react. (Only three such antiserums against Pearlneck cells have been produced in more than 200 rabbits during a period of more than 15 years.) Hence it has been necessary to combine two or more antiserums containing different combinations of antibodies, or at other times to use in the tests an antiserum to another species, chiefly to Pearlneck. Some of the antigens peculiar to either Pearlneck or Senegal appear to be weakly antigenic—only seldom do they induce the formation of antibodies—so it has been simply a matter of expediency to use whatever antiserum would react with a particular antigen. The peculiarities of the response of different rabbits to immunization with the same batch of Pearlneck cells have been described (IRWIN and GOLDEN 1942), and are more or less typical of each series of immunizations with any kind of cells. The reasons for the differential response of the rabbits are unknown at present; if heritable differences are operative, there is no known method by which they may be controlled.

EXPERIMENTAL RESULTS

Segregation of Cellular Antigens Specific to Senegal

Using the distribution of the antigenic complexes specific to Senegal, which have been observed in the first backcross generation (*loc. cit.*), as a point of departure, after several further backcrosses it was possible to identify as units a majority, if not all, of the segregating antigens. Each cellular component was considered to be a unit when, in backcrosses to Ring dove, the progeny of any bird containing it were in two categories—(a) those with and (b) those without the component. These different substances have been temporarily designated as *s-1*, *s-2*, *s-3*, *s-6*, *s-7*, *s-8*, *s-9*, *s-10*, *s-11*, and *s-12* ("*s*" refers to Senegal). Those at one time called *s-4* and *s-5* were not reproduced in one case and were found to be a duplicate in the other, so these two numbers are not included in the numerical sequence. The immunological relationships of these different characters are given in table 1.

As was stated above, certain antigens have not reacted with more than a few of the antiserums. While the summary in table 1 is the result of tests with Senegal antiserum, actually it is a compilation of tests with several antiserums to Senegal, and of one to Pearlneck. The *s-9* character only rarely reacted even weakly with a Senegal antiserum, and many of the tests for individuality between it and other substances of Senegal were made with Pearlneck antiserum.

The quantitative expression of each of these unit characters with the "reagent" for Senegal substances (Senegal antiserum absorbed only by Ring dove cells) is given in the second column of the table. Numerous tests with several different antiserums are combined in this column. It will be noted that the *s-1* character reacted at the fifth or sixth dilutions of antiserum; that is, at a dilution of 1:1440 or 1:2880 in the mixture of antiserum and cells. The antigens *s-1*, *s-2*, *s-7*, *s-8* and *s-12* were uniformly the strongest to react, although

antibodies to *s-7* and *s-12* appeared in only about half the antiserums. Antibodies in usable potency to one or more of the respective antigens, *s-3, s-6, s-9, s-10, and s-11*, appeared occasionally in antiserums in various combinations. Only rarely were all present in one antiserum.

TABLE 1

Agglutination interactions of the cellular antigens specific to Senegal.

RED BLOOD CELLS		AGGLUTINATION REACTIONS OF RED BLOOD CELLS CARRYING THE UNIT ANTIGENS OF SENEGAL LISTED IN THE FIRST COLUMN TO THE LEFT, WITH SENEGAL ANTISERUM ABSORBED EITHER BY RING DOVE CELLS ALONE, OR BY CELLS OF RING DOVE PLUS THOSE OF BACKCROSS BIRDS CONTAINING, RESPECTIVELY, ONE OF THE SENEGAL CHARACTERS LISTED BELOW IN THE HORIZONTAL ROW									
		s-1	s-2	s-3	s-6	s-7	s-8	s-9	s-10	s-11	s-12
Ring dove	o	o	o	o	o.	o	o	o	o	o	o
Senegal	8, 9	9		9	9	9	9	9	9	9	9
s-1	5, 6	o	++	++	++	++	++	++	++	++	++
s-2	6	++	o	++	+	++	++	++	++	++	++
s-3	3	±	±	o	±	+	+	+	+	+	+
s-6	2, 3	+	+	+	o	+	+	±	+	+	±
s-7	4, 5, 6	++	++	+	++	o	++	++	++	+	++
s-8	6, 8	++	++	++	++	++	o	++	++	++	++
s-9	2, 3	±		±	±	±	±	o	±	±	±
s-10	3, 4	+	±	±	±	++	+	+	o	+	±
s-11	2, 3	+	+	++	+	+	+	+	+	o	+
s-12	4, 5	++	±	+	++	+	++	+	++	++	o

The digits represent the highest dilution of serum at which agglutination was visible microscopically. For example, if 1=60, 2=120, 3=240 . . . 9=15,360.

Symbols: ++ or +=strong or definite agglutination; ±=weak but positive agglutination; o=no agglutination—all at the first serum dilution.

That each of these antigens may be distinguished from the others can be deduced from the results given in table 1. The basic assumption that each antigenic substance induces the formation of antibodies specific to itself can be tested by removing the antibodies from the immune serum, not only those to the complex of Ring dove cells but also to each of the presumed unit substances. Any absorbed serum so obtained is then tested against the cells of all the other unit-substances and should cause them to agglutinate.

For example, Senegal antiserum absorbed only by Ring dove cells becomes a "reagent" for the antigens of Senegal cells which are not in Ring dove. This reagent may be made more specific by including the cells of Ring dove and those carrying *s-1* in the absorption procedure, and then testing the resulting fluid or reagent with cells carrying the various unit-substances. As is shown in the third column in table 1, this reagent did not agglutinate the cells of either Ring dove or those possessing the *s-1* character (the specific antibodies for such cells have been removed), but did agglutinate the other unit-characters

of Senegal. Similarly, each of the other nine components of Senegal cells, when used in absorption of Senegal antiserum in combination with Ring dove cells, removed antibodies specific only to itself and reacted with cells carrying all the other fractions of the specific Senegal complex. The reagent from which the antibodies to *s-2* have been removed (fourth column in table 1) has not been tested for interaction with cells containing *s-9*, but there is no reason to doubt the outcome of the interaction. These specificities of reaction as given in table 1 allow the conclusion to be made that there are at least ten antigenic characters in the cells of Senegal, as are listed in table 1, which distinguish it from Ring dove.

It is probably unnecessary to state that all birds which supposedly contained any antigenic character in unit-form were compared, each with several others, for identity of antigenic composition. That is, the cells of any bird which might contain *s-8* were compared immunologically in cross-tests with the cells of other birds with that antigen. If the unknown cells removed the antibodies for antigen *s-8* from Senegal antiserum, and were not agglutinated by reagents from which the antibodies for *s-8* alone had been exhausted, the antigen in the cells was indistinguishable from *s-8* and was therefore presumed to be identical. Whenever possible, the cells of each individual were tested reciprocally with those of the backcross hybrid parent, in order to determine if the antigen were present *in toto* or only in part. The same kind of procedure was followed for each of the various antigens now proposed as units.

No association of any one of these antigens with either sex or morphological character has been observed. Each of the antigens has assorted independently of sex and the "dark" color of the Senegal, which behaves as if it were homologous with the dark sex-linked color of the Pearlneck (COLE 1930).

The different antigenic substances were also tested genetically for unity, in backcrosses to Ring dove of birds containing each antigen. The outcome of these tests is given in table 2. As stated above, it is expected that birds possessing only one of these antigens should produce from such matings only two kinds of offspring in approximate equality; namely, (a) those with and (b) those without the antigen.

This expectation is realized in each of the different tests, as is shown in table 2. Admittedly the number of offspring from birds possessing *s-2*, *s-6* and *s-10*, respectively, is extremely small. The backcross hybrids carrying either *s-2* or *s-6* died without leaving offspring, so it has been necessary to attempt to reisolate these two antigens from birds of the first backcross generation; a process which after three years of effort gives promise of success. The character *s-10* appears to be lost, with no hope of obtaining it again except by a repetition of the original procedure. Since there is no immediate prospect of adding to the numbers of progeny from these three characters, the data are presented with the numbers as given.

The distribution of the offspring with or without the antigen can most reasonably be explained in each case as approximating that expected if but one genetic character were involved. Other data on the distribution of single antigens, but from backcrosses in which each unit substance was in combina-

tion with one or two others (as *s-7* with *s-8*, or *s-6*, *s-7* and *s-12* together) are in complete accord with the thesis that each is heritable and is produced independently. Such data, however, will not be published but are permanently filed in the Editorial Office of GENETICS.

Segregation of Antigens Specific to Ring Dove

Following matings of the species hybrids to Senegal, a segregation in the progeny of cellular components specific to Ring dove would be expected. Seldom, however, under our laboratory conditions do female Senegals or female species hybrids lay eggs; hence offspring from the above backcross would be much more difficult to obtain than from the backcrosses to Ring dove.

TABLE 2

Distribution of the antigens specific to Senegal (of table 1) in the progeny from matings to Ring dove of backcross birds possessing only one of the respective cellular substances.

SENEGAL SPECIFIC SUBSTANCE	NUMBER OF BACKCROSS OFFSPRING WITH	
	SUBSTANCE PRESENT	SUBSTANCE ABSENT
S-1	35	33
S-2	1	3
S-3	24	25
S-6	1	3
S-7	18	26
S-8	14	25
S-9	13	7
S-10	1	3
S-11	43	35
S-12	13	11

However, 18 individuals of the first and seven of the second backcross generation have been produced. Their cells have been available at one time or another—never all at the same time—for tests of the segregation of antigenic components specific to Ring dove. Efforts to produce more backcross offspring have been quite unsuccessful.

A definite segregation of antigens peculiar to Ring dove was noted in the cross-tests which were made with the cells of the various individuals. However, the publication of these various interactions seems unwarranted. The data are permanently filed in the Editorial Office of GENETICS. An accurate estimate cannot be made of the number of substances involved which are peculiar to Ring dove, although at least five would be required in explanation of some of the interactions. Presumably, but not necessarily, approximately the same number differentiate Ring dove from Senegal as have been noted in Senegal in comparison with Ring dove. The pertinent point is that the same kind of segregation was observed in the first backcross generation of matings to Senegal and, given the proper set of environmental conditions for the Senegals

to produce offspring, it would have been possible to isolate the specific antigens of Ring dove in unit form.

DISCUSSION

The picture of the antigenic relationship between the blood cells of Senegal and Ring dove parallels very closely that previously observed between Pearlneck and Ring dove. That is, one or more genes on at least ten chromosomes of Senegal have antigenic effects which differentiate Senegal from Ring dove, as was found to be the case in the comparison of Pearlneck and Ring dove (IRWIN 1939). Nearly all the backcross hybrids in which these antigens have been obtained as single entities are descendants of a mating between one Senegal male and a Ring dove female (producing family D376), as previously described (IRWIN and COLE 1945a). The second isolation of characters *s-2* and *s-6*, as mentioned previously, is from species hybrids (family E153) resulting from another mating. No two of these antigenic characters have behaved as if they were a contrasting pair in Senegal; i.e., one or the other antigen was not found in the respective species hybrids. Also no fractionation of these specific characters of Senegal has been observed to date, as would be expected if two or more genes produced each character and crossing over took place between the causative genes. However, unpublished evidence is available to the effect that fractionation of one, possibly of two of the antigens specific to Pearlneck (in contrast with Ring dove) infrequently occurs, suggesting crossing over between genes which are involved in their production. Both antigens of Pearlneck are closely related to certain of those of Senegal. Because of these observations, and other considerations which will be dealt with more fully in a paper to follow, it appears to the authors to be quite probable that at least certain, perhaps all, of these antigenic substances of Senegal are produced by the joint action of two or more genes.

To these ten chromosomes of Senegal carrying one or more genes with effects on the cellular antigens specific to the species must be added the three or four with effects on the antigens of the serum (CUMLEY, *et al.*, 1943). Therefore, at least 13 or 14 of a total of about 30 chromosome pairs of Senegal—this being the best estimate to date of the number of chromosomes of species of pigeons and doves (PAINTER and COLE, 1943)—carry one or more genes with antigenic effects differentiating it from Ring dove. Although slightly less than half the number of chromosome pairs is involved in this differentiation of Senegal from Ring dove, one cannot at present distinguish which chromosomes—large or small—carry the genes with antigenic effects. Therefore a guess as to the proportion of chromatin material involved would be premature.

Thus the relationship between Pearlneck and Ring dove on one hand, and Senegal and Ring dove on the other, are more or less parallel. It remains to be seen to what extent the unit antigens of Pearlneck and Senegal are alike. These studies will be presented elsewhere.

SUMMARY

The data presented show that there are at least ten antigenic characters in the red blood cells of Senegal which distinguish the species from Ring dove.

Each of these cellular components behaves in inheritance as if it were produced by one or more genes on a particular chromosome of Senegal. Thus there are at least ten chromosomes of Senegal carrying a gene or genes with antigenic effects differentiating it from Ring dove. A segregation of antigens specific to Ring dove in accordance with genetic expectancy was also observed but an estimate of the total number involved is not possible.

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THE APPEARANCE OF A BALANCED LETHAL SITUATION IN THE OENOTHERA HETEROZYGOTE GAUDENS·^{h}FRANCISCANA FOLLOWING SEVERE HEAT TREATMENT OF SEEDS[1]

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THIS paper will give the history of a line of Oenothera heterozygotes following the substitution in the cytoplasm of *Lamarckiana* of the ^{h}franciscana complex for the velans complex of *Lamarckiana*. The heterozygote gaudens·^{h}franciscana is of course the laeta plant of the twin hybrids from the cross *Lamarckiana*×*franciscana*. The velutina plant of the twins has the constitution velans·^{h}franciscana.

The heterozygote gaudens·^{h}franciscana gives a plant phenotypically very close to *Lamarckiana* and reproduces itself with each generation but always throws a large class of narrow-leaved etiolated seedlings that die early under the usual conditions of cultivation. The etiolated seedlings are ^{h}franciscana·^{h}franciscana with the two sets of chromosomes in the cytoplasm of *Lamarckiana*.

The heterozygote gaudens·^{h}franciscana, therefore, behaves genetically like gaudens·velans of *Lamarckiana* in reproducing itself and in failing to give gaudens·gaudens, which is zygote lethal, but the ^{h}franciscana·^{h}franciscana segregate is not zygote lethal as is velans·velans although represented by physiologically very weak plants. Text figure 1 shows a pan of 100 seedlings from an F_8 generation of a line of gaudens·^{h}franciscana; there were 36 dark-green, broader-leaved, vigorous gaudens·^{h}franciscana and 64 etiolated. narrower-leaved, weak ^{h}franciscana·^{h}franciscana.

In a number of generations of this structural hybrid gaudens·^{h}franciscana experiments were introduced to test the effects of very severe heat treatment of dry seeds in the hope that such treatment might bring about the elimination of the class of etiolated seedlings through the appearance and establishment of a zygote lethal. A race did arise following heat treatment that ceased to throw the class of etiolated seedlings at the same time presenting a corresponding decrease in the percentage of fertile seeds. A zygote lethal appeared in the ^{h}franciscana complex and the heterozygote gaudens·^{h}franciscana attained through a mutation the balanced lethal situation characteristic of *Lamarckiana*. Of course one record such as this does not prove that the mutation was induced by the heat treatment, but let the conditions and the results of the experiments speak for its probability.

With this account, as not inappropriate, there is included under Section 2

[1] Genetical Studies on Oenothera XX. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 831.

data on the F_2 of velans·^{h}franciscana (cytoplasm of *Lamarckiana*) and the F_2 of ^{h}franciscana·velans (cytoplasm of *franciscana*), and on back crosses of the F_1 plants to *franciscana*. This inclusion will not seriously break the main account which is treated in the following steps: Section 1. The cross *Lamarckiana*×*franciscana* and its reciprocal. Section 3. The F_2, F_3 and F_4 generations of gaudens·^{h}franciscana to the time of severe heat treatment of dry seeds. Section 4. The method of heat treatment applied to seeds from which were grown the F_5 and F_6 generations. Section 5. The F_7, F_8, and F_9 generations, large cultures with lower seed viability which gave no etiolated plants, indicating the establishment of a balanced lethal situation. Section 6. Discussion.

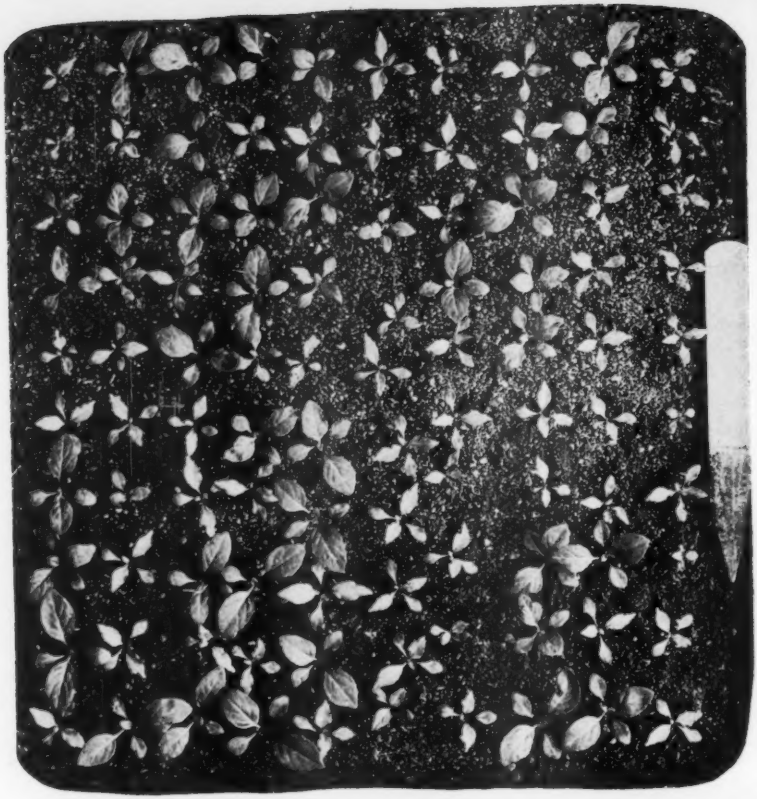
1. THE CROSS LAMARCKIANA×FRANCISCANA AND ITS RECIPROCAL

A culture (36.43) of the cross *Lamarckiana*×*franciscana*, germination 85.4 percent, gave 62 gaudens·^{h}franciscana (laeta twin) and 102 velans·^{h}franciscana (velutina twin), the latter a group of more or less etiolated and weak plants of which more than one-half died early and only 11 plants matured. These two types are the twin hybrids identified by DE VRIES (1918, p. 406) as laeta and velutina following studies on the cross *Lamarckiana*×*Hookeri* (DE VRIES 1913, p. 113, 1918) where similar products result because *Hookeri* and *franciscana* are closely related species. The twin hybrids carry respectively with the chromosome sets of *franciscana* the two gamete complexes of *Lamarckiana* named by RENNER (1916, 1917) gaudens and velans.

The chief points of contrast between the twin hybrids are:

	gaudens·^{h}franciscana (laeta twin)	velans·^{h}franciscana (velutina twin)
Seedlings and young rosettes	Leaves dark-green, broad. Plants strong.	Leaves partly or wholly etiolated, narrow. Plants weak, the most strongly etiolated dying early.
Mature rosettes	Leaves dark-green, broad, much crinkled. Strong.	Surviving rosettes with leaves gray-green, narrower, less crinkled.
Mature plants	Leaves dark-green. On the stems, ovate, sessile, crinkled.	Leaves gray-green. On the stems lanceolate, short-petioled, almost plane.
Buds	9.5 cm. Cones 4.7 cm. Sepals green, light-red streaks, no red papillae. Hypanthium green.	8–9 cm. Cones 3.8 cm., stout. Sepals light-red, narrow green central streak, red papillae. Hypanthium reddish.
Sepal tips	8–9 mm, slender, separating, acute-pointed.	5 mm, thick, appressed, blunt.
Capsules	23–25 mm, green.	28–30 mm, reddish.
Pollen	30–60 percent good.	95–99 percent good.

The presence of the gaudens complex in gaudens·^{h}franciscana, cytoplasm of *Lamarckiana*, gives a plant with broad, dark-green, crinkled leaves; sepal tips slender, separating, acute pointed; pollen about 50 percent good. The



FIGURES 1 AND 2. (*For explanation see page 187*)

plant is scarcely distinguishable from *Lamarckiana*. It does, however, have stigmas only 4-lobed (*Lamarckiana* 4, 5, 6 and 7, lobed), capsules 23–25 mm long (*Lamarckiana* about 20 mm), and petals a lighter shade of yellow as in *franciscana*.

A franciscana set of chromosomes with velans giving velans·^{h}franciscana in the cytoplasm of *Lamarckiana* makes possible viable zygotes in contrast to the zygote lethal velans·velans from *Lamarckiana*. The velans·^{h}franciscana zygotes, however, produce etiolated plants so weak that most of them die as seedlings and of the surviving plants set in the field very few reach maturity. The plants of velans·^{h}franciscana that live (in contrast to gaudens·^{h}franciscana) have narrower, gray-green, almost plane leaves; sepal tips thick, appressed, and blunt; capsules longer; pollen about 95 percent good. The surviving plants are similar to *franciscana* and frequently somewhat larger.

The chromosome configuration of the F_1 gaudens·^{h}franciscana (36.43-28) is a circle or chain of 10 chromosomes and 2 pairs, and of the F_1 velans·^{h}franciscana (36.43-70) a circle or chain of 4 chromosomes and 5 pairs, in agreement with findings of CLELAND (1932, p. 587, 595). The parent *franciscana* of my line carries 7 pairs of chromosomes as tested in 1928, 1933, and 1936. The gaudens·^{h}franciscana thus shows one less segmental interchange than does *Lamarckiana* with its circle of 12 chromosomes and one pair.

The reciprocal cross *franciscana*×*Lamarckiana* (culture 36.44), germination 72.4 percent, gave 10 ^{h}franciscana·gaudens (laeta twin) and 97 ^{h}franciscana·velans (velutina twin). The chromosome complexes of each type in the reciprocal operate of course in the cytoplasm of *franciscana*. The plant of ^{h}franciscana·gaudens appears quite the same as gaudens·^{h}franciscana, the character of the cytoplasm, whether *Lamarckiana* or *franciscana*, having no important effect on the expression of the plant's characters. The cytoplasm of *franciscana*, however, proved to be a more favorable medium for ^{h}franciscana·velans than the cytoplasm of *Lamarckiana* for velans·^{h}franciscana since no etiolated seedlings appeared, the 97 plants of ^{h}franciscana·velans being rather larger and more vigorous than *franciscana* plants.

The chromosome configuration in ^{h}franciscana·gaudens (36.44-100), as would be expected, proved to be a circle or chain of 10 and 2 pairs as in gaudens·^{h}franciscana. It may be assumed that ^{h}franciscana·velans (CLELAND 1932, p. 588) will show the same configuration as velans·^{h}franciscana, a circle of 4 and 5 pairs. However, the plant of ^{h}franciscana·velans (36.44-17) tested in this culture presented a circle of 6 and 4 pairs indicating a further segmental

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EXPLANATION FOR FIGURES 1 AND 2

FIGURE 1.—Pan of 100 seedlings from an F_2 generation of a line of gaudens·^{h}franciscana. There were 36 dark-green, broad-leaved, vigorous gaudens·^{h}franciscana and 64 etiolated, narrow-leaved, weak ^{h}franciscana·^{h}franciscana.

FIGURE 2.—Plant 40.58-12 of gaudens·^{h}franciscana showing the habit, foliage, slender bud cones, and large flowers typical of *Lamarckiana*. A seed of this plant after severe heat treatment gave plant 41.67-3 in which a zygote lethal appeared in the franciscana chromosome complex eliminating in further line breeding the class of etiolated ^{h}franciscana·^{h}franciscana segregates.

interchange in this particular plant. These findings agree with records of EMERSON and STURTEVANT (1931, p. 398).

As will be reported in Section 2 there were plants of velans·^{h}franciscana in the F_2 which showed occasional pollen mother cells with chromosome arrangements a circle of 6 and 4 pairs among cells with the much more common circle of 4 and 5 pairs. Occasional circles of 6 and 4 pairs also appeared in velans·^{h}franciscana plants of the back cross (velans·^{h}franciscana)×*franciscana*. There is clearly a tendency in both velans·^{h}franciscana and ^{h}franciscana·velans towards an additional segmental interchange to give circles of 6 and 4 pairs.

2. DATA ON THE F_2 OF VELANS·^{h}FRANCISCANA AND THE F_2 OF ^{h}FRANCISCANA·VELANS, AND ON BACK CROSSES OF THE F_1 PLANTS TO FRANCISCANA

An F_2 generation (37.47) was grown from one of the 11 plants (36.43-70) of the velutina class that matured in the F_1 *Lamarckiana*×*franciscana* of the original cross (36.43). This type velans·^{h}franciscana in the cytoplasm of *Lamarckiana*, as stated in Section 1, is similar to *franciscana*, but its chromosome arrangement at diakinesis is a circle of 4 chromosomes and 5 pairs (*franciscana* parent 7 pairs). The plant with full expression of viability would be expected to segregate 2 velans·^{h}franciscana:1 ^{h}franciscana·^{h}franciscana (velans·velans being zygote lethal). From the culture of 143 plants, germination 69.1 percent, 127 matured. These fell into two groups (1) 44 plants of velans·^{h}franciscana, broader leaves, stouter bud cones, later flowering; chromosome arrangement, tested for two plants (37.47-97, 37.47-132) generally a circle of 4 and 5 pairs but with occasional circles of 6 and 4 pairs, and (2) 85 plants of ^{h}franciscana·^{h}franciscana, narrower leaves, more slender bud cones, earlier flowering; chromosome arrangement, tested for one plant (37.47-22), 7 pairs. A more robust habit and later flowering readily distinguished the velans·^{h}franciscana plants from the altogether typical *franciscana* segregates, but in numbers they were far below expectations. The cytoplasm of *Lamarckiana* in this culture (37.47) was not unfavorable for a strong development of the segregates, but it should be noted, as stated in Section 1, that the parent F_1 velans·^{h}franciscana (36.45-70) was one of only 11 plants surviving from a group of 102 more or less etiolated and weak plants of the velutina class in the original cross *Lamarckiana*×*franciscana*. In the DISCUSSION will be found some consideration of RENNER'S interesting hypothesis to account for the presence of chlorophyll in these 11 plants.

An F_2 generation (37.49) was grown from the interesting reciprocal F_1 plant ^{h}franciscana·velans (36.44-17), cytoplasm of *franciscana*, which presented the peculiarity of a chromosome configuration with a circle of 6 and 4 pairs (as noted in Section 1) instead of the expected circle of 4 and 5 pairs. From this F_2 culture of 149 plants, germination 76.7 percent, 139 matured, 40 distinguished as ^{h}franciscana·velans and 99 recognized as ^{h}franciscana·^{h}franciscana, again a segregation with a very much lower representation of ^{h}franciscana·velans than would be expected.

Data are given below on back crosses to the *franciscana* parent of the two F_1 hybrids velans·^{h}franciscana and ^{h}franciscana·velans. The plants grown to maturity were random selections from young rosettes of large cultures.

Culture 37.63 (velans·^{h}franciscana)×*franciscana* gave 6 velans·^{h}franciscana and 161 *franciscana*, all green.

Culture 37.64 *franciscana*×(velans·^{h}franciscana) gave 4 ^{h}franciscana·vellans and 46 *franciscana*, all green.

Culture 37.67 (^{h}franciscana·velans)×*franciscana* gave 7 velans·^{h}franciscana and 43 *franciscana*, all green.

Culture 37.68 *franciscana*×(^{h}franciscana·velans) gave 11 ^{h}franciscana·vellans and 38 *franciscana*, all green.

In culture 37.63 chromosome configuration was determined for two plants of velans·^{h}franciscana (37.63-96 and 37.63-107) showing circles of 4 and 5 pairs with occasional circles of 6 and 4 pairs, and, also, for one plant of the *franciscana* segregate (37.63-42) showing 7 pairs. The results from these back crosses supported the findings of the segregation from the F_2 generations that in numbers the hybrids were far below expectations showing that some differential worked against their appearance.

3. THE F_2, F_3, AND F_4 GENERATIONS OF GAUDENS·^{h}FRANCISCANA, TO THE TIME OF SEVERE HEAT TREATMENT OF DRY SEEDS

The F_2, F_3, and F_4 generations from the heterozygote gaudens·^{h}franciscana (cytoplasm of *Lamarckiana*) followed the same pattern to the time when severe heat treatment of dry seeds was introduced in the F_5 generation. Each generation threw the two classes (1) dark-green, strong gaudens·^{h}franciscana (*Lamarckiana*-like) and (2) etiolated, weak ^{h}franciscana·^{h}franciscana. The history is outlined in table 1.

If there were no gamete or zygote lethals, or other factors to modify fertility, the plant gaudens·^{h}franciscana would of course be expected to give a progeny of 1 gaudens·gaudens, 2 gaudens·^{h}franciscana, and 1 ^{h}franciscana·^{h}franciscana. The zygote lethal of gaudens eliminates gaudens·gaudens as in the breeding of *Lamarckiana*, but ^{h}franciscana does not have a zygote lethal and the segregate ^{h}franciscana·^{h}franciscana in the cytoplasm of *Lamarckiana* is represented by a large class of etiolated seedlings. Why the seedlings are not green and vigorous has perhaps some relation to the cytoplasm of *Lamarckiana* in which the two sets of *franciscana* chromosomes lie. This problem will receive some consideration in the DISCUSSION. There were many more of the etiolated ^{h}franciscana·^{h}franciscana in each generation than of the dark-green gaudens·^{h}franciscana indicating the presence of factors cutting down the production of the latter.

The plants gaudens·^{h}franciscana in the F_2 were similar to *Lamarckiana* except, as noted before, the stigmas were 4-lobed, the capsules longer, and the petals a lighter shade of yellow. In the F_3 generation among the 44 plants that matured one was noted (38.92-41) bearing a few flowers with stigmas 5 and 6-lobed. The line was continued from this plant and all later generations gave plants with the variable number of stigma lobes characteristic of *Lamarckiana*.

In the line of gaudens·^{h}franciscana through its 9 generations no plants have appeared with the short capsules (20 mm) of *Lamarckiana* or with petals of its darker shade of yellow. Many examinations of pollen from gaudens·^{h}franciscana showed it to be from 50 to 60 percent good as in *Lamarckiana*.

TABLE 1

Pedigree of the line gaudens·^{h}franciscana in which, following severe heat treatment of dry seeds, a balanced lethal situation appeared.

CULTURE	PARENT PLANT	SEEDLINGS	% GERMINATION	GAUDENS·^{h}FRANCISCANA	^{h}FRANCISCANA·^{h}FRANCISCANA	HEAT TREATMENT OF DRY SEEDS
F_2 37.45	36.43-28	514	70.7	102 dark-green seedlings produced 71 mature plants, 20 rosettes, 11 died	412 etiolated seedlings produced 33 mature plants, 379 died	No heat treatment
F_3 38.92	37.45-13	315	88.4	67 dark-green seedlings produced 44 mature plants, 9 rosettes, 14 died	248 etiolated seedlings produced 41 mature plants, 207 died	Seeds of 37.45-13 at 85°C for 2 days. Treatment not severe enough to lower the high percentage of seed germination
F_4 39.51	38.92-41	264	56.5 A test 87.04	60 dark-green seedlings produced 26 mature plants, 33 rosettes, 1 died	204 etiolated seedlings produced only 6 plants able to survive and mature in the greenhouse	No heat treatment
F_5 40.58	39.51-1	61	44.2	12 dark-green seedlings produced 10 mature plants and 2 rosettes A mortality of 28 seedlings that failed to survive the operation for seed coat removal	21 etiolated seedlings died early	Seeds of 39.51-1 at 90°C for 6 days. This severe heat treatment greatly lowered the percentage of seed germination
F_6 41.67	40.58-12	36	16.5	10 dark-green seedlings produced 10 mature plants A mortality of 23 seedlings that failed to survive the operation for seed coat removal	3 etiolated seedlings died early	Seeds of 40.58-12 at 90°C for 5 days. Percentage of seed germination very much reduced
F_7 42.51	41.67-3	414	39.89 Tests 36.2 33.97	414 dark-green seedlings produced 274 mature plants, 63 rosettes, 77 died in the field or earlier	No etiolated seedlings	No heat treatment
F_8 43.51	42.51-53	119	29.02 A test 35.4	119 dark-green seedlings produced 86 mature plants, 16 rosettes, 3 nanella-like, 14 died	No etiolated seedlings	No heat treatment
F_9 44.51	43.51-8	177	47.3	177 dark-green seedlings produced 159 mature plants, 5 rosettes, 6 narrow-leaved dwarfs, 7 died	No etiolated seedlings	No heat treatment

Chromosome configuration in gaudens·^{h}franciscana was recorded for the following plants in the history of its cultivation, as outlined in table 1, F_1 36.43-28, F_2 37.45-13, F_3 38.92-41, F_4 39.51-1, F_7 42.51-53. There was no departure from the chromosome arrangement noted in the F_1, namely a circle or chain of 10 chromosomes and 2 pairs.

The plants ^{h}franciscana·^{h}franciscana (cytoplasm of *Lamarckiana*) were always etiolated as seedlings, the basal half of the cotyledons and the following

two leaves being light yellow. Most of the seedlings developed no green of consequence and died early. A few produced later leaves more or less light green in color, and some plants in the F_2 and F_3 matured in the field. In the F_4 generation (table 1) none of the 204 etiolated seedlings produced plants strong enough to set in the field, but seven were nursed in the green house of which six matured. All of the 80 plants from etiolated seedlings in the F_2, F_3, and F_4 that matured showed the striking peculiarities of adult *franciscana*—a foliage of gray-green leaves; sepals light-red; sepal tips short, thick, appressed, blunt; capsules long (28–30 mm); pollen examined from many plants about 95 percent good. The chromosome configuration was studied in two plants of ^{h}franciscana·^{h}franciscana (38.93-60, 38.93-66) from an F_3 generation of a line parallel to that given in table 1. The pollen mother cells in diakinesis showed 7 pairs with striking clearness in agreement with my line of *franciscana*.

4. THE METHOD OF HEAT TREATMENT APPLIED TO SEEDS AND THE RESULTS SHOWN IN THE F_5 AND F_6 GENERATIONS

Heat treatment of seeds in a rough way accomplishes quickly one of the results of the aging of seeds when kept from moisture, that is the removal of some of the water contained within the embryo and its surrounding tissues. The treatment was planned to be so severe that almost all of the seeds of a particular lot treated would be killed. From surviving seeds cultures were grown and plants of gaudens·^{h}franciscana were selfed. After a period of ripening, assisted by exposure to cold, samplings from such seed were tested for the percentages of their germination. If the germination percentage of the seeds from any plant fell markedly below that usual for gaudens·^{h}franciscana (around 80 percent) then it might be hoped that a zygote lethal had appeared in the ^{h}franciscana complex suppressing the development of fertile seeds of ^{h}franciscana·^{h}franciscana. Finally, cultures from such seeds would determine whether or not the etiolated seedlings of the class ^{h}franciscana·^{h}franciscana had been eliminated from the breeding.

If later generations in selfed line showed the complete disappearance of of ^{h}franciscana·^{h}franciscana from the progeny, accompanied by a low percentage of germination, it would be fair to conclude that a zygote lethal had developed in the ^{h}franciscana complex (lethal in double dose). Since the gaudens complex has such a zygote lethal there will have appeared a race of gaudens·^{h}franciscana breeding true through the suppression of gaudens·gaudens and ^{h}franciscana·^{h}franciscana, both being represented by abortive seeds. This would be the balanced lethal situation of *Lamarckiana* interpreted by MULLER (1918).

The technique of the heat treatment was simple. Seeds in open vials were grouped about the bulb of a thermometer inserted from above into a dry oven kept at a constant temperature by a thermostat regulating heat from electric bulbs. However, it took many months of experimentation to determine how great was the resistance of the seeds both to the degree of heat and to the length of treatment. The recording of this experimentation would take many pages; the main results can be stated shortly.

As the severity of the heat treatment is increased by raising the temperature and lengthening the period of treatment there comes a point at which most of the seeds will be killed and this is shown by an abrupt fall in the percentage of germination from such treated seeds. Some seeds of the line of gaudens·^{h}franciscana given in table 1 lived through a temperature of 90°C for 6 days (culture 40.58), a surprising performance. At temperatures above 90°C death came much more quickly, and heat of 95°C kills in a few hours. Prolonged heat treatment hardens the seed coats making the germination more difficult and operations with the point of a scalpel are frequently necessary to release seedlings unable to draw themselves free from their seed coats; there is high mortality from this operation. Thus in culture 40.58 (table 1) 28 seedlings failed to survive the operation of seed coat removal and a ratio of the dark green seedlings to the etiolated could not be determined, but from a number of tests the evidence is strong that the seeds of the etiolated ^{h}franciscana·^{h}franciscana are less resistant to the higher temperatures over the longer periods of time than are the seeds of the dark green gaudens·^{h}franciscana.

In the line of gaudens·^{h}franciscana given in table 1 heat was first applied in the F_3 generation (culture 38.92) but this treatment of 85°C for 2 days proved to be too light to lower the fertility of the treated seeds expressed by 88.4 percent seed germination. The F_4 generation showed no marked change over the F_2 and F_3.

This brings us to the F_5 generation when the first severe heat treatment in this line was applied to the seeds of the parent plant 39.51-1 in the F_4. Heat at 90°C for 6 days killed directly about half of the seeds (since the percent of germination fell to 44.2), and rendered 28 seedlings impotent to free themselves from their seed coats. There matured 10 plants (culture 40.58) a' gaudens·^{h}franciscana and three of these were self-pollinated. The germination percentages of seeds from these three plants as tested were all high (40.58-1, 70.3 percent; 40.58-2, 71.4 percent; 40.58-12, 81.6 percent) and it was decided to proceed further with heat treatment. Seeds of the plant 40.58-12, text figure 2, were selected for this further heat treatment because an abundant harvest offered opportunity for experimentation.

Seeds from the F_6 plant 40.58-12 selfed were subjected to several experimental degrees of heat treatment and from one treatment reducing mortality to a 16.5 percent germination after heat of 90°C for 5 days there was grown the F_6 generation, 41.67 of table 1. Culture 41.67 produced 10 dark-green seedlings, 3 etiolated seedlings, and there was a mortality of 23 seedlings from among those failing to survive the operation of removal from the seed coats. The low germination of 16.5 percent and the survival of only 10 plants from the 218 seed-like structures with which the culture started indicates the severity of the heat treatment. All of the 10 dark-green seedlings matured plants of gaudens^{h}franciscana; all were selfed.

The seeds of these 10 plants of gaudens·^{h}franciscana in the F_6 were then subjected to a preliminary test for percentage of seed germination, and to determine the character of the seedlings with results as given in table 2. Except for the seeds from plant 41.67-3 the germination percentages were high

(71.3 to 86.1) and the seedlings were of the two classes (1) dark-green, strong gaudens·^{h}franciscana and (2) etiolated, weak ^{h}franciscana·^{h}franciscana. The etiolated seedlings were in much smaller ratios to the dark-green than in the earlier generations of the line (compare with the F_2, F_3, and F_4 given in table 1). Occasional "empty" seeds contained small amounts of material.

TABLE 2

Results from tests applied to the 10 plants of F_6 gaudens·^{h}franciscana in culture 41.67 (table 1) for percentage of seed germination and to determine the character of the seedlings.

PLANT	GERMINATIONS	EMPTY SEEDS	PERCENT OF GERMINATION	SEEDLINGS DARK-GREEN AND STRONG	SEEDLINGS ETIOLATED AND WEAK	DIED BEFORE CHARACTER COULD BE DETERMINED
41.67-1	185	41	81.8	80	96	9
41.67-2	147	39	79	63	82	2
41.67-3	152	268	36.2	145	None	7
41.67-4	145	45	76.3	64	79	2
41.67-5	172	33	83.9	75	79	18
41.67-6	160	34	82.5	61	73	26
41.67-7	152	49	75.6	69	81	2
41.67-8	144	58	71.3	75	66	3
41.67-9	166	42	79.8	66	96	4
41.67-10	192	31	86.1	81	103	8

Plant 41.67-3, table 2, in its performance stood out in the group of 10 plants with striking contrasts. The germination percentage of its seeds was only 36.2 and the 145 seedlings were all dark-green and strong; there were no etiolated weak seedlings. This plant then of low seed fertility which produced no etiolated seedlings satisfied the conditions indicative of the presence of the balanced lethal situation desired. There remained the testing of this plant and its progeny in line cultures and this has been done through three generations, the F_7, F_8, and F_9 of table 1, as described in the next section.

5. THE F_7, F_8, AND F_9 GENERATIONS, LARGE CULTURES WITH LOW SEED VIABILITY WHICH GAVE NO ETIOLATED PLANTS, INDICATING THE ESTABLISHMENT OF A BALANCED LETHAL SITUATION

At this point it is well to give the peculiarities of the F_6 plant 41.67-3 gaudens·^{h}franciscana (parent of the line with balanced lethals) distinguishing it from the line of *Lamarckiana* used in the original cross. As noted in Section 3 the stigmas which were 4-lobed in the F_1 and F_2 became 4, 5, or 6-lobed, as in *Lamarckiana*, in the F_3 plant 38.92–41. However, the capsules (23–25 mm) remained longer than in *Lamarckiana* and the petals continued to be the lighter shade of yellow characteristic of the *franciscana* parent. These distinctions continued unmodified through the F_7, F_8, and F_9 generations and seem to be securely fixed in the line. In other respects gaudens·^{h}franciscana is a good replica of *Lamarckiana* giving evidence that *Oenothera franciscana* must be very close to a hypothetical velans·velans without the zygote lethal.

The F_7 generation (table 1) was the first generation of the line with balanced lethals. Culture 42.51 (from plant 41.67-3) germination 39.89 percent, was a large culture of 414 dark-green seedlings which produced 274 mature plants and 63 large rosettes; 77 young plants died. All plants were of the gaudens·^{h}franciscana type. There were no etiolated seedlings.

The F_8 generation (table 1) was the second generation in the line of balanced lethals. Culture 43.51 (from plant 42.51-53) germination 29.02 percent (a test gave 35.4 percent), started with 119 dark-green seedlings and produced 86 mature plants and 16 large rosettes of gaudens·^{h}franciscana; 14 plants died. No etiolated seedlings. There were also 3 *nanella*-dwarfs in the culture and one of these selfed gave in the following year a culture (44.52) of 157 dark-green seedlings, germination 38.4 percent, from which 128 produced dwarfs similar to the parent; 29 plants died. Since there was no class of etiolated seedlings the *nanella*-like dwarf must have carried the balanced lethal mechanism of its parent. In this behavior the *nanella*-like dwarf followed that of *nanella* from *Lamarckiana*. Plant 42.51-53, parent of the F_8 generation, showed the chromosome configuration of four earlier plants in the line (36.43-28, 37.45-13, 38.92-41, 39.51-1) a circle or chain of ten and two pairs.

The F_9 generation, the third generation in the line with balanced lethals, was represented by cultures grown in the green house from four different plants in the F_8 and by one culture grown in the field (table 1). Those grown in the green house were as follows: (1) Culture 44.101 (from 43.51-8); 395 seedlings, germination 46.2 percent, produced 388 dark-green, broad-leaved, strong rosettes and five narrow-leaved green rosettes also strong but dwarf. There were no etiolated seedlings. Of the five green dwarf rosettes two sent up central shoots producing narrow-leaved dwarfs about four dm high with large flowers, and buds with sepal tips slender, separating, and acute pointed as in gaudens·^{h}franciscana. From one of these narrow-leaved dwarfs (44.101-1) a culture of 151 plants (46.101) was grown in the green house, germination 38.2 percent, no etiolated seedlings; 125 narrow-leaved dwarfs matured with the characters of the parent plant thus indicating it to be a dwarf mutant from gaudens·^{h}franciscana and with the balanced lethal mechanism. (2) Culture 44.102 (from 43.51-41); 139 seedlings, germination 36.6 percent, gave a culture of 139 rosettes all dark-green, broad-leaved and strong; no etiolated seedlings. (3) Culture 44.103 (from 43.51-48); 161 seedlings, germination 34 percent, developed into 161 dark-green, broad-leaved, strong rosettes; no etiolated seedlings. (4) Culture 44.104 (from 43.51-100); 166 seedlings, germination 40.6 percent, produced 166 rosettes dark green, broad-leaved and strong; no etiolated seedlings. Thus four different plants from the F_8 culture 43.51 gave cultures with low percentages of germination and no etiolated seedlings; the dark green strong rosettes were undoubtedly those of gaudens·^{h}franciscana.

A culture 44.51 (table 1) from seed of the same plant (43.51-8) that gave culture 44.101 in the greenhouse was grown in the field. There were 177 seedlings, germination 47.3 percent, all dark green and strong, none etiolated. All seedlings lived and of the 177 rosettes set out 159 matured as plants of gaudens·^{h}franciscana, five remained rosettes, six became narrow-leaved dwarfs

and seven died. Of the six dwarfs only two developed shoots and flowered; these had large flowers and sepal tips slender, separating, and acute pointed as in gaudens·^{h}franciscana.

In summary of the data on the F_9 generation a total of 1036 seedlings were carried through the rosette stage or farther. None of the seedlings and rosettes were etiolated or weak; all were dark-green and strong. The germinations were below 50 percent. In the one culture grown in the field all of the 159 plants that matured were gaudens·^{h}franciscana.

These findings for the F_7, F_8, and F_9 generations give the evidence for the conclusions: (1) That a zygote lethal appeared in the *franciscana* chromosome complex of a seed from the F_5 plant 40.58-12 following severe heat treatment. (2) That this lethal was destructive in double dose in the F_6 plant 41.67-3 grown from this seed with the result that the franciscana segregates (etiolated seedlings) from gaudens·^{h}franciscana dropped out in the selfed line from plant 41.67-3. (3) That therefore a modified line of gaudens·^{h}franciscana arose with a balanced lethal mechanism which breeds true (except for mutations) for the same reasons that gaudens·velans (*Lamarckiana*) breeds true.

6. DISCUSSION

Oenothera is remarkable among plant groups in having developed to a very high degree peculiarities dependent upon two evolutionary principles in cytology and genetics.

First. Through segmental interchanges chromosomes associate at diakinesis end to end in chains to form circles of four or higher even numbers up to a possible circle of 14 chromosomes. Alternate chromosomes in such circles are segregated at meiosis and therefore in hybrids with circles of 14 chromosomes (and with smaller circles when the free pairing chromosomes are identical) there will be produced no more than two types of gametes. Almost invariably in the larger circles there is present the balanced lethal mechanism. A circle of 14 chromosomes was first reported and described by CLELAND in 1923 and 1926 for *muricata* and recently CLELAND (1944, p. 8) reports studies on more than 100 geographical races (biotypes) from the Rocky Mountain region and eastward in the United States and Canada, all self-pollinating and all with circles of 14 chromosomes and balanced lethals.

Second. These circle forming Oenothera hybrids segregating no more than two types of gametes breed true through the operation of lethals under two conditions: (1) Gamete lethals destroying one chromosome complex in the sperm and the other complex in the egg will give self-perpetuating complex-heterozygotes. (2) Zygote lethals, fatal in double dose, will elininate the homozygous segregates from a complex-heterozygote and leave only the heterozygote able to live and to reproduce the hybrid. Such zygote lethals are termed "balanced" (MULLER 1918) and to operate they must be in different loci of synaptic mates (when they are known to cross over), or they may lie in chromosomes of a circle that pass each to a different gamete. Very little is known of the position of zygote lethals in the chromosome circles of Oenothera.

It is surprising that other groups of plants have not to a greater extent developed through segmental interchange circle-forming chromosome complexes. Small circles of four or six are known for several much studied genera (Datura, Zea, Pisum, Campanula, Rhoea, etc.), but rarely are all of the chromosomes included in a single circle. Moreover, why are balanced lethal mechanisms rarely developed in these genera?

Since *Oenothera franciscana* is very closely related to *O. Hookeri* consideration must be given to the findings of DE VRIES (1918) and RENNER (1924, 1934) on chlorophyll color in crosses between *Hookeri* and *Lamarckiana*, and to the interpretation of RENNER. The cross *Lamarckiana*×*Hookeri* gives fully green gaudens·^{h}Hookeri (laeta twin) and etiolated weak velans·^{h}Hookeri (velutina twin) most of which die as seedlings or young rosettes. Some of the etiolated plants, however, may develop green areas on the leaves or even become light green plants and may mature. In this cross the chromosome complexes operate in the cytoplasm of *Lamarckiana*. The reciprocal cross *Hookeri*×*Lamarckiana* gives ^{h}Hookeri·gaudens (laeta) and ^{h}Hookeri·velans (velutina) both fully green, the chromosome complexes operating in the cytoplasm of *Hookeri*. This breeding behavior is paralled by that reported in this paper for the cross *Lamarckiana*×*franciscana* and its reciprocal.

RENNER assumed that *Lamarckiana* plastids in the cytoplasm of *Lamarckiana* function and multiply with a gaudens·^{h}Hookeri chromosome complex but not with a velans·^{h}Hookeri chromosome complex. The few mottled or light-green plants among the strongly etiolated velans·^{h}Hookeri in the cytoplasm of *Lamarckiana* were interpreted by RENNER as taking their green color from Hookeri plastids brought by the pollen tube into the cytoplasm of *Lamarckiana*. This interpretation would seem to assume that a gaudens chromosome complex is necessary for the functioning of *Lamarckiana* plastids. In the reciprocal cross *Hookeri*×*Lamarckiana* the *Hookeri* chloroplasts operate normally because they are in *Hookeri* cytoplasm with a Hookeri chromosome complex and, therefore, both of the twin hybrids ^{h}*Hookeri*·gaudens and ^{h}Hookeri·velans are fully green. RENNER's hypothesis possibly does not give full consideration to the problem of why *Hookeri* plastids assumed to be introduced through the pollen tube into *Lamarckiana* cytoplasm in velans·^{h}Hookeri should find in such cytoplasm a favorable medium for their activities. Let us examine the problem from this angle.

Because *Lamarckiana* is a complex-heterozygote the bulk of its cytoplasm may have come from either one of two putative parents, an assumed gaudens·gaudens or an assumed velans·velans, depending upon whether the original cross was gaudens·velans or velans·gaudens. The true nature of the cytoplasm and plastids of *Lamarckiana* might then be either chiefly or wholly of a gaudens or a velans parentage. What might be the interpretation of the results of chlorophyll inheritance fitted into the above assumptions? In our discussion we shall now substitute *franciscana* for *Hookeri* since the two biotypes are closely related and their breeding behavior with *Lamarckiana* is the same, as noted by DE VRIES (1918), HOEPPENER and RENNER (1929), and STURTEVANT (1931).

Assuming that the cytoplasm of *Lamarckiana* is gaudens cytoplasm then *Lamarckiana*×*franciscana* would give gaudens·^{h}franciscana (green laeta) and velans·^{h}franciscana (etiolated velutina), both in cytoplasm gaudens. The green gaudens·^{h}franciscana would have one set of gaudens chromosomes, the etiolated velans·^{h}franciscana no gaudens chromosomes. If the presence of at least one set of gaudens chromosomes is necessary for the development of chlorophyll in gaudens cytoplasm the green laeta is understandable, but on this hypothesis the velutina twin, velans^{h}franciscana, in gaudens cytoplasm should not be green and yet the facts are that occasionally partially etiolated plants are found. RENNER's hypothesis that the pollen tube at times brings in functioning plastids must assume that *Hookeri* and *franciscana* plastids either will produce chlorophyll in a gaudens cytoplasm or that the entering plastids are accompanied by enough of their own cytoplasm to make possible their activities to some degree. It is the latter possibility that I would propose and stress.

Assuming that *Lamarckiana* has velans cytoplasm with velans chloroplasts, then *Lamarckiana*×*franciscana* would give a green gaudens·^{h}franciscana (laeta) because the *franciscana* chromosome complex is so similar to that of velans that its substitution for velans would not interfere with the production of chlorophyll. The velutina twin, velans·^{h}franciscana, in velans cytoplasm would most certainly be green since velans plastids are in their own cytoplasm and ^{h}franciscana is similar to velans. However, the facts are that the velutina twin is etiolated. So the assumption that *Lamarckiana* has velans cytoplasm is ruled out.

Considering the reciprocal cross *franciscana*×*Lamarckiana* with *franciscana* cytoplasm in the egg both ^{h}franciscana·gaudens and ^{h}franciscana·velans would be green (which they are) because each of the twin hybrids has a set of *franciscana* chromosomes friendly to the operation of *franciscana* plastids.

Let us examine the breeding record of our plant gaudens·^{h}franciscana (table 1) on the assumption that the functioning of gaudens plastids in gaudens cytoplasm requires an accompanying gaudens chromosome complex. For three generations (F_2, F_3, and F_4) the plant threw consistently etiolated seedlings (^{h}franciscana·^{h}franciscana) in numbers three to four times greater than the dark-green parent type. While almost all of the etiolated seedlings died early there were always in each generation a few light-green plants that lived in the field or could be nursed to maturity in the green house. To explain on RENNER's hypothesis the survival of green franciscana segregates in gaudens cytoplasm we must either admit some tolerance by franciscana plastids of gaudens cytoplasm or assume some favorable modification of such cytoplasm, perhaps through the introduction of *franciscana* cytoplasm with the *franciscana* plastids from the pollen tube.

It would seem probable that the genetic character of cytoplasm may at times limit the activities of chloroplasts, producing conditions of metabolic interference with their normal activities. Thus etiolation may in some cases be tied up with genes, cytoplasm, and plastids in a relationship far from simple. There are other cases of etiolated segregates in Oenothera genetics that deserve study from this point of view.

There is no evidence through the F_2, F_3, and F_4 generations (table 1) of a marked running out of the etiolated seedlings or of any strengthening of their position as segregates. Their ratios to the dark green seedlings in these generations were respectively F_2, 412:102 (4:1), F_3 248:67 (3.7:1), F_4 204:60 (3.4:1). This is important since it, therefore, appears clear that the mutation which took place in the F_6 generation giving plant 41.67-3 with its balanced lethal mechanism could have no connection with the degree of etiolation or the size of the class of etiolated segregates, ·^{h}franciscana·^{h}franciscana, which is eliminated. The lethal gene must lie in a franciscana chromosome and it is a zygote lethal since after its establishment no etiolated seedlings appeared in the F_7, F_8, and F_9 generations.

SUMMARY

The cross *Lamarckiana*×*franciscana* gives the twin hybrids gaudens·^{h}franciscana (laeta) and velans·^{h}franciscana (velutina). Since the chromosome sets of ^{h}franciscana and velans are closely related the plant gaudens·^{h}franciscana is similar to *Lamarckiana* (gaudens·velans). When grown in selfed lines the plant reproduces itself and behaves like *Lamarckiana* in failing to give gaudens ·gaudens (zygote lethal), but differs from *Lamarckiana* in throwing a large class of etiolated seedlings which are ^{h}franciscana·^{h}franciscana in the cytoplasm of *Lamarckiana* and which take the place of velans·velans (zygote lethal) in the breeding of *Lamarckiana*.

Seeds of gaudens·^{h}franciscana were exposed to dry heat at high temperatures for long periods in the hope of inducing a zygote lethal into the franciscana chromosome complex that would operate like the lethal in velans and in double dose eliminate the class of etiolated segregates ^{h}franciscana·^{h}franciscana. Heat of 95°C killed seeds rather quickly but some seeds could survive an exposure for six days to heat of 90°C.

In an F_6 generation of gaudens·^{h}franciscana from dry seeds subjected to heat of 90°C for five days a culture, germination 16.5 percent, gave ten plants of gaudens·^{h}franciscana. Seeds from nine of these plants selfed showed the normal high germination of around 80 percent, but seeds from one plant (41.67-3) gave a low germination of only 36.2 percent (table 2). From this plant the selfed line was continued through large F_7, F_8, and F_9 generations with the low percentage of germination maintained and with a complete elimination from the line of etiolated seedlings representing ^{h}franciscana·^{h}franciscana (table 1).

The breeding of the line thus became that of *Lamarckiana*, i.e. zygote lethals suppressed the appearance of gaudens·gaudens and ^{h}franciscana·^{h}franciscana. Except for certain dwarf mutants (also with a zygote lethal in ^{h}franciscana) the line bred true for the complex-heterozygote gaudens·^{h}franciscana. A balanced lethal mechanism developed in association with severe heat treatment of dry seeds although on the strength of one case it would not be safe to assume that heat alone was responsible for the appearance of the zygote lethal.

The chromosome configuration at diakinesis of gaudens·^{h}franciscana is a

circle or chain of 10 chromosomes and 2 pairs, thus showing one less segmental interchange than in *Lamarckiana*. The configuration in velans·^{h}franciscana is a circle of 4 chromosomes and of 5 pairs. The reciprocal cross ^{h}franciscana·gaudens, as would be expected, showed a circle of 10 chromosomes and 2 pairs. However, in ^{h}franciscana·velans there appeared cases of an additional segmental interchange to give circles of 6 and 4 pairs. Plants of velans·^{h}franciscana and of ^{h}franciscana·velans were larger and later in flowering than in *franciscana*; otherwise they were similar.

A few plants of the ^{h}franciscana·^{h}franciscana segregate developed enough chlorophyll to live in the field or in the green house. Those that matured showed the characters of *franciscana*—gray-green foliage; sepals light-red; sepal tips short, blunt, thick, appressed; petals lemon-yellow; capsules long (28–30) mm; pollen 95 percent good. There were 7 pairs of chromosomes at diakinesis as in the parent *franciscana*.

In the DISCUSSION will be found some comments on the problem of chlorophyll inheritance presented by the class of etiolated segregates from gaudens·^{h}franciscana. RENNER'S interpretation of the presence of some green plants among the etiolated is reviewed. It is suggested that the history of the cytoplasm in the complex-heterozygotes of Oenothera becomes important to the understanding of problems of their etiolated segregates. It seems probable that the interrelations between chromosome complexes and the genetic character of the cytoplasm may at times limit the activities of chloroplasts, producing conditions of metabolic interference with their normal functions.

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A VARIABLE PHENOTYPE ASSOCIATED WITH THE FOURTH CHROMOSOME OF *DROSOPHILA MELANOGASTER* AND AFFECTED BY HETEROCHROMATIN*

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ORIGIN

THE character called "sparkling" (*spa*) was first seen in some of the scute bobbed (*sc bb*) females of a stock of closed X (X^{cl}) heterozygous for *sc bb*. Two *sc bb* virgin females having rough eyes were mated separately each to three *sc bb* males from the same mass culture of *sc bb*/X^{cl} (the males of the stock, as is usual, did not show *bb* because of the presence of its wild-type allele in the Y chromosome). One female produced sparkling females, all of which were also *sc* and *bb*, and males which were all *sc* and not-*bb* and were not-*spa*.

The second female produced *sc bb; spa* females, and *sc* not-*spa* females, the latter all *bb* except one which was rated as an XXY female. The males were *sc* not-*bb* not-*spa* except one in which both *bb* and *spa* were manifested to an exaggerated degree. The exception judged by the exaggeration of *bb* was undoubtedly an XO male.

In the first culture the manifestation of sparkling accompanied that of the recessive sex-linked character bobbed, but in the second culture though all of the F_1 females were bobbed many of them were not-*spa*. The mother had been *spa* so that if *spa* were a recessive character, it had not been carried in the X chromosome of her mate. The two classes of females, wild-type and *spa*, in F_1 suggested that the father might have been heterozygous for a recessive autosomal gene whose expression in the F_1 males was suppressed in the presence of a Y chromosome. The exceptional XXY female (not-*bb* and not-*spa*) and the exceptional XO male (exaggerated *bb* and exaggerated *spa*), were in accord with the interpretation.

Females homozygous for *sc bb; spa* were mated to males carrying the dominant mutant Curly (*Cy*) in chromosome 2 and Stubble (*Sb*) in chromosome 3, and others were mated to males that were shaven naked (sv^n) a recessive mutant in chromosome 4; *spa* did not appear in the F_1 generation. In F_2 females, *spa* showed independent assortment with *Cy* and with *Sb*, but none of the F_2 *sv* females was *spa; spa* was thus shown to be a recessive character associated with a differential in the largely heterochromatic fourth chromosome. The region associated with the expression of *spa* was determined by use of the translocation T(2; 4)b in which the break has been found by E. B. LEWIS (unpublished) to be distal to the proximal bands of 102C (BRIDGES' salivary chromosome map, 1935). In crosses between *spa* and the translocation, males and females survived that carried a normal second chromosome from each

* The cost of the accompanying tables is paid by the GALTON and MENDEL MEMORIAL FUND.

parent, one fourth chromosome from the parent that was *spa* and the 4-2L fragment which was composed of the centromere and proximal region of chromosome 4, as far as to include the proximal region of 102C, and of the tip of 2L. The hyperploid (for 2) is recognized as a short plump fly with small scutellum, broad thorax, small spread wings and small sometimes knotty legs and bulging eyes. Dp(2; 4)b flies both female and male that carried the single fourth chromosome showed a very high degree of *spa*, indicating that the altered region of the chromosome which is in some way associated with *spa* is distal to nearly all of region 102C. In salivary glands of larvae heterozygous for *spa* there appears a disturbance in the distal range of 102C, proximal to 102D1. The character and the extent (perhaps variable) of the disturbance have not been determined.

By genetic methods *spa* is found to be related to the dominant mutant Cataract (*Cat*) in the fourth chromosome discovered by BELGOVSKY (unpublished). *Cat* is recognized as a roughness and thickening of the posterior rim (or half) of the eye visible in both males and females. In flies heterozygous for *Cat* and *spa* the anterior part of the eye is slightly *spa* in XX females and decidedly *spa* in XO males.

From the first matings of *spa* it had been found that its manifestation was in some way correlated with the presence and absence of the Y chromosome but not in the sense that *bb*, which was present with *spa*, is suppressed by Y. The normal allele of *bb* in Y accounts for the latter whereas *spa* was found to be associated with a differential located in the fourth chromosome. The effect of Y on *spa* suggested rather the suppression of mosaicism in the class of position effects which are known as variegations, such as Plum (*Pm*) in the second chromosome, the white variegated series in the X and numerous others.

The eye of sparkling presents no visible mosaic pattern as is seen in roughest (*rst*^{3}, KAUFMANN 1942) or in eye color variegations, but the grade of roughness in individuals varies among the progeny of pairs of flies as does the extent of mosaicism shown by individuals in populations of variegations. With this lead experiments were made to test *spa* for other properties of variegations, namely, differences in the extent of expression at different temperatures and reaction to differing amounts of heterochromatin in the nucleus; also incidental observations were made bearing on the detection of a possible maternal effect of heterochromatin.

The eyes of homozygous sparkling females are often somewhat bulging. The ommatidia appear to vary in convexity and to vary in size so that their boundaries form uneven lines giving a disturbed appearance to the surface of the eye. The posterior rim of a slightly *spa* eye may be somewhat angular ventrally rather than smoothly curved. In low grades of *spa* the rough effect varies if the eye is turned to receive the light at different points. A sparkling effect may result from uneven illumination of the irregular ommatidia and from reflection of light from the hairs between, which seem to be longer than normal; with increased degree of *spa* the glistening hairs may project well beyond the surface of the eye when viewed from above. In extreme *spa* no dark fleck or rays are seen, and the eye is often small and condensed (ommatidia

sometimes unevenly crowded); although a high degree of *spa* may occur also in large eyes (differences characteristic of different lines).

In order to facilitate classification an arbitrary scale of seven degrees of *spa* was chosen. An eye of grade 6 is exceedingly rough, has no fleck, and is often bulging, round, and small. At the other extreme, in grade 1, roughness is very slight, may appear not to involve all of the eye and is sometimes discernible only when the eye is seen in certain positions in relation to the source of light; it scarcely differs from grade 0 or smooth. Grade 2 is evidently sparkling and three more steps (grades 3–5) lead to the extreme, grade 6. When feasible, one parent of the matings was heterozygous for *spa* and for its wild-type allele in a chromosome marked by the dominant wing character, cubitus interruptus dominant (ci^D). Thus homozygous *spa* eyes could be compared in the same conditions directly with wild-type eyes which sometimes give the appearance of a surface which is granular rather than entirely smooth.

The counts of ci^D flies have not been entered in the tables. The ci^D flies have been found very useful for direct comparison in classification of grades 0 and 1 although even so the separation probably is not entirely accurate; in no case has there been any decided roughness in eyes of ci^D flies. In exp. 371 the eyes of ci^D looked granular and the sixteen $\underline{XX}Y$ females classed as grade 1 may have been grade 0.

As there is no actual division between grades, the classification of borderline cases varies. Various factors may cause roughness of eye and for this reason normal controls were present whenever possible in the same cultures in which *spa* was rated in flies of altered genetic constitution, or sibs were used as P_1 flies.

The flies were raised at about 18°C except those in a few experiments where a higher temperature is noted in the tables. Three of these were controls for the effect of temperature.

In the description of matings the females are mentioned first. About three to five females were usually used in one culture, or in some experiments only one pair of flies, and the results of the separate cultures were summated.

The frequency distribution of grades of *spa* in eyes of flies of normal constitution is shown in table 2 and on the right of table 3. It may be seen on inspection that in every experiment except 131a, 131l (table 3), and 58bi (table 2) there is one mode of distribution and that the expression of *spa* was greater in females than in males. The percentage of females above grade 3 has been chosen as the distribution index of frequencies of grades of *spa* in females of normal constitution and the percentage over grade 1 as the index for normal males (table 5). The index for males is often lower than the index for females; although it is based on a frequency which includes two grades lower than the base of the female index. It is noticeable also that a shift in distribution is always in the same direction in both sexes and the modes of distribution vary correspondingly (table 5). In 1944 the smoothest flies and the most extreme *spa* of the two bimodal lines mentioned above were segregated, and in 1946 there are two nearly pure lines differing in the expression of *spa*, from each one of the original lines. There is also a third instance of a line (no. 102) of

high degree of *spa* which was separated in 1944 from smoother flies. These had been used in a test of effect of age on the expression of *spa*. The flies were graded daily for six days and they showed no effect of age. The distribution did not give two modes, but the high frequencies overlapped three successive

TABLE 1

F_1 homozygous spa (at 18°±, 37l at 22°±). All parental fourth chromosomes spa unless denoted as ci^D. 119m,* X-X-Y, *y sc $bb^{Df\,G}$; ci^D×y sc $bb^{Df\,G}$. 53B,* X-X-Y, *y sc $bb^{Df\,G}$×y sc $bb^{Df\,G}$; ci^D. 87B, same. 53A,* XXY, bb×*y sc $bb^{Df\,G}$. 105p,* XXY bb; *ci^D×y sc $bb^{Df\,G}$; ci^D. 37l,* XXY bb; *ci^D×y sc $bb^{Df\,G}$; ci^D. 37p,* XXY ♀×*y sc $bb^{Df\,G}$; ci^D. 114a,* XXO bb×(*bb*?). *114j,* XXO bb×*sc bb. 114k,* XXO bb×*bb.*

NO.	P_1 ♀	F_1	0	1	2	3	4	5	6
119m	X-X-Y	X-X-Y	42	48	2	—	—	—	—
		X-Y	—	2	33	43	16	3	—
		X-YY	64	—	—	—	—	—	—
53B	X-X-Y	X-Y	—	—	20	21	3	—	—
		X-YY	42	—	—	—	—	—	—
87B	X-X-Y	X-X-Y	19	28	—	—	—	—	—
		X-Y	—	—	—	49	54	—	—
		X-YY	66	—	—	—	—	—	—
53A	XXY	XXY	11	(XXO	—	—	—	17	12)
		X-Y	—	—	—	26	34	—	—
105p	XXY	XXY	55	(XXO	—	—	1	5)	—
		X-Y	—	—	1	8	27	7	—
		X-YY	5	(XXO†	—	—	1	11)	—
37l*	XXY	XXY	24	16	(XXO	1	3	2)	—
		X-Y	—	6	20	52	13	—	—
		X-YY	16	(XXO†	—	—	12	4)	—
37p	XXY	XXY	62	(XXO	—	—	3	7	4)
		X-Y	—	—	4	30	35	5	—
		X-YY	3	—	—	—	—	—	—
114a	XXO	XXY	14	—	—	—	—	—	—
		XO	(2XY)	—	—	—	—	14	10
114j	XXO	XXY	40	—	—	—	—	—	—
		XO	—	—	—	—	—	6	22
114k	XXO	XXY	69	—	—	—	—	—	—
		XO	—	—	—	—	—	26	21

† Additional XXO females.

grades (2 to 4). In the highest grades were two kinds of males not easily separable. The eyes of one kind were small, slightly lighter red, and less uniformly rough. These males were chosen as the male parents which produced the rougher line. A test of the high line derived from 58bi for a new factor for roughness not in the fourth chromosome gave a negative result. The other lines were not tested.

In some strains of *spa* which were obtained from exceptions among progeny of X-rayed males (MORGAN, REDFIELD, and MORGAN 1943) the eyes are smooth, in others the eyes are extreme *spa* both in males and in females. These phenotypes are recessive to wild type.

The distribution indices of normal genotypes (table 5) are widely different, varying from 0 percent to 100 percent for females and from 0 to 72 percent (in four experiments 79 to 98 percent) for males. Many of the higher indices were obtained from populations into which *spa* had just been introduced with

TABLE 2

F_1 homozygous spa and samples of stock [74], [a], etc. (at 18° ±, at 22 ± marked, at 25° marked†). All parental fourth chromosomes spa unless denoted as ci^D or ey^D. 58a, sc bb [74]. 103a, sc bb [ext.]. 59a, sc bb; ci^D [a]. 121a, (bb?); ci^D×(bb?). 121l, (bb?)×(bb?). 120a, (bb?); ey^D×(bb?). 120l, (bb?)×(bb?). 58l, sc bb; ci^D [a]. 58s, sc bb; ci^D [a]. 23a, sc bb [a]. 23t,† same. 58bi,* sc bb [75]. 58y*, sc bb [75^A]. 58z*, sc bb [75^I]. 57l, XXY bb×sc bb. 57v, XXY f×sc bb; ci^D.*

NO.	F_1	0	1	2	3	4	5	6
58a	XX	4	6	51	63	16	—	—
	XY	72	23	6	1	—	—	—
103a	XX	—	—	24	29	19	9	—
	XY	26	38	10	11	3	—	—
59a	XX	—	—	—	9	74	8	—
	XY	—	27	58	38	3	—	—
121a	XX	—	—	3	11	32	20	—
	XY	1	13	19	6	9	2	1
121l	XX	—	—	—	—	32	163	—
	XY	—	16	47	67	29	2	1
120a	XX	—	—	—	3	30	5	—
	XY	3	23	23	6	—	—	—
120l	XX	—	—	—	9	55	95	—
	XY	4	38	36	26	17	—	—
58l	XX	—	—	—	—	—	23	—
	XY	1	2	4	16	12	—	—
58s*	XX	—	—	—	10	—	—	—
	XY	10	—	—	—	—	—	—
23a	XX	—	—	3	39	111	25	—
	XY	—	2	81	77	16	2	—
23t†	XX	2	31	106	37	3	—	—
	XY	162	29	1	—	—	—	—
58bi*	XX	—	1	4	12	3	8	14
	XY	16	3	—	—	9	15	3
58y*	XX	—	—	—	—	2	5	13
	XY	—	—	—	1	3	7	9
58z*	XX	1	5	4	5	5	—	—
	XY	4	9	3	2	1	—	—
57l	XY	—	69	67	7	—	—	—
57v	XY	4	58	78	18	—	—	—

some other chromosome, such as deficiency for chromosome 2 or 4, or ci^D and ey^D (eyeless dominant) in exps. 121a, 121l, 120a, 120l. In making the matings the more extreme *spa* flies were probably selected from the stocks from which they were chosen. Some of them may have represented a new extreme line rather than a sample from the higher grades of a uniform line.

EFFECT OF TEMPERATURE ON THE EXPRESSION OF *spa*

A high degree of *spa* had been noted whenever flies had been raised at low temperatures, but the wide range of modes of distribution of flies of normal constitution (cf. tables 2 and 5, right of table 3) makes it clear that the effect of a factor under investigation can be judged only by the most complete control. A sample of flies of stock (a) raised at about 22°C (table 2, exp. 58s) consisted of ten females of grade 3 and ten smooth males. A sample raised at the same time at about 18°C (exp. 58l) consisted of 23 females of grade 5 and 35 males of which 91 percent were above grade 3. About two years later a larger sample of the same line of flies was raised at about 19° (table 2, exp. 23a) and a control at 25° (exp. 23t) and gave similar results.

Two relations are evident. At both temperatures females show a higher degree of *spa* than do the males of the same populations, and at the lower temperature there is a shift toward more extreme *spa* in both sexes.

Exp. 23t shows an aggregation of 85 percent of the males in grade 0; these are all the males below the highest two grades in which any males occur. In exp. 23a the males below the highest two grades, in this case 90 percent of males, are distributed among three classes. This must mean that beyond the threshold of what is called normal (that is, the grade which appears in the wild-type fly) there is, at 25°, variation in the degrees of *spa* which would have been visibly expressed if the flies had been raised at 19°. It would then follow that when a maximum frequency is found in grade 0, the mode is beyond the threshold of normal and is not detectable. Accordingly, the grade of the mode cannot be determined (cf. tables 4 and 5). It may be that there are undetectable characteristics of the *spa* eye which are an expression of grades of *spa* beyond the normal threshold at 25°, just as at 19° differences are visible among the smoother grades.

THE EFFECTS OF DIFFERENT AMOUNTS OF HETEROCHROMATIN IN THE NUCLEUS

Sensitivity to amounts of heterochromatin in the nucleus which is shown by *spa* and by the variegation type of position effect (NOUJDIN 1939) was studied in different genotypes. The pronounced effect of low temperature on the expression of *spa* was utilized in experiments involving various amounts of heterochromatin. There was an advantage in raising the flies at 17° to 19° because *spa* would be visibly expressed in a greater number of flies and because rougher eyes are more easily classified than smoother.

It had been shown by the progeny of the first matings of sparkling flies which were also bobbed that the difference in the expression of *spa* in females and males was not, as in the case of *bb*, due to a normal allele in the Y chromosome; a differential of some kind associated with the character *spa* was found to be located in the fourth chromosome. The effect of Y might resemble the effect found in variegations which is correlated with the ratio of heterochromatin to euchromatin in the chromosome complement. Accordingly, the degrees of *spa* were measured when different amounts of heterochromatin and euchromatin were present in the sex chromosomes or in an autosome.

TABLE 3

F_1 homozygous spa; left, modified genotypes, right, normal genotypes in the same population (at 18°± except 119k and 90t* at 22°±). All parental fourth chromosomes spa unless denoted as ci^D, Df 4 or o. 130A, Dp(1;f) 101/y^2 cv v(bb?)/y^2 cv v(bb?); $ci^D \times y^2$ cv v(bb?); ci^D. 11a, same. 11m, y^2 cv v(bb?)×y^2 cv v(bb?). 104k, y sc $bb^{Df\,G}$/bb; $ci^D \times bb$. 104n, same. 94a, same. 94k, bb; $ci^D \times bb$; ci^D. 119k, bb; $ci^D \times y$ sc bb^{Df}; ci^D.*

NO.	P_1 ♀	F_1	o	1	2	3	4	5	6
130A	$X^{Dp}XX$	$X^{Dp}XX$	—	25	42	7	3	—	—
		$X^{Dp}XY$	19	(Sample)	—	1	—	1	—
11a	$X^{Dp}XX$	$X^{Dp}XX$	—	—	50	27	2	—	—
11m	XX	—							
104k	X-X	X-X	—	—	—	11	35	39	—
		X-Y	2	25	49	9	—	—	—
104n	X-X	X-Y	2	11	10	—	—	—	—
		—							
94a	X-X	X-X	—	—	—	—	16	38	29
		X-Y	—	49	55	—	—	—	—
94k	XX	—							
		—							
119k*	XX	X-X	—	—	1	5	15	7	—
90a	XX	XY^{bbl}	—	—	—	—	—	7	—
90t*	XX	XY^{bbl}	—	—	10	8	6	6	1
109	$2^-/2$	$XX2^-/2$	—	—	—	—	—	30	17
		$XY2^-/2$	—	—	—	1	11	24	10
131a	$2^-/2$	$XX2^-/2$	1	4	14	19	10	37	77
		$XY2^-/2$	4	8	4	8	11	25	89
131l	2/2	$XX2^-/2$	1	1	6	7	17	5	43
		$XY2^-/2$	2	6	6	4	1	15	25
125a	$4^-/4$	$XX4^-/4$	19	—	—	—	—	—	—
		$XY4^-/4$	20	—	—	—	—	—	—
128a	$4^-/4$	—							
		—							
128l	4/4	—							
		—							
114w	4/o	XX4/o	—	—	—	—	—	15	—
		XY4/o	—	—	26	30	11	3	—
114v	4/4	XX4/o	—	—	—	—	—	27	—
		XY4/o	—	1	22	43	23	3	—
129a	4/o	XX4/o	—	—	—	—	—	—	6
		XY4/o	—	—	—	—	1	5	—
129l	4/4	XX4/o	—	—	—	—	—	4	13
		XY4/o	—	—	—	4	30	15	1

Females carrying a Y and males without a Y chromosome were obtained in the same experiments (table 1, exps. 114a, j, k) among the progeny of attached-X females without a Y (XXO). Such females were daughters of attached-X homozygous *bb* females (XXY) mated to males in which nondisjunction was frequent; the character *bb* when visible in the females indicated the absence of the Y chromosome. Their XXY daughters were 100 percent normal, and the XO sons were of the highest grades of *spa*, 5 and 6 (some of the males were genetically *bb* and these showed exaggerated *bb*). The addition of Y to

TABLE 3

(*Continued*)

90a, sc bb×sc bb/Y^{bbl}. 90t, same. 109, Df(2) Ms10 ci^{D}. 131a, Df(2) Ms10×not-Df. 131l, not-Df ×Df(2) Ms10. 125a, Df(4) M-4×spa. 128a, same. 128l, spa×Df(4) M-4. 114w, (bb?); spa/o ×(bb?). 114v, (bb?)×(bb?); spa/o. 129a, spa/o×spa. 129l, spa×spa/o.

NO.	P_1 ♀	F_1	0	1	2	3	4	5	6
130A	$X^{Dp}XX$	XX	—	—	11	45	31	3	—
		XY	36	1	2	1	(Sample)		(1 XO?)
11a	$X^{Dp}XX$	XX	—	—	13	46	16	2	—
11m	XX	XX	(1 XXY?)	—	16	90	35	11	—
104k	X⁻X	XX	—	2	29	37	20	1	—
		—							
104n	X⁻X	XY	78	1	—	—	—	—	(2 XO?)
		—							
94a	X⁻X	XX	—	4	13	33	52	14	3
		XY	70	8	—	—	—	—	—
94k	XX	XX	—	—	—	8	33	18	2
		XY	40	17	1	—	—	—	—
119k*	XX	XY	11	—	—	—	1	—	(2 XO?)
90a	XX	XX	—	—	—	—	—	75	1
90t*	XX	XX	—	—	1	2	13	10	—
109	2⁻/2	XX	—	1	7	19	8	1	—
		XY	9	11	5	—	—	(1 XO?)	—
131a	2⁻/2	XX	17	41	33	20	33	25	6
		XY	2	59	47	15	8	11	6
131l	2/2	XX	17	14	18	16	19	6	—
		XY	36	9	9	1	2	—	—
125a	4⁻/4	XX	—	—	2	16	20	2	—
		XY	6	19	15	—	—	—	—
128a	4⁻/4	XX	—	—	6	18	42	54	3
		XY	13	34	53	27	11	6	—
128l	4/4	XX	2	3	8	22	31	39	4
		XY	23	29	36	17	7	6	2
114w	4/o	XX	—	—	35	17	—	—	—
		XY	42	1	—	—	—	—	—
114v	4/4	XX	—	5	20	10	4	—	—
		XY	29	10	—	—	—	—	—
129a	4/o	XX	—	—	—	27	19	1	—
		XY	6	9	23	—	—	—	—
129l	4/4	XX	1	3	34	36	30	9	—
		XY	19	20	5	—	—	—	—

XX in females suppressed *spa*, and in the XO males which lacked a Y the expression of *spa* was decidedly more extreme than in XY males.

It is to be noted that XXO females show a high degree of *spa*. This was observed in exps. 53A, 105p, 37l, and 37p (table 1, 37l at 22°) and in the stock which was examined at the same time. All of the XXO females were of grade 4 at least and usually even higher. The attached-X combination is deficient in heterochromatin due to loss of the right arms of the two X's and due perhaps also to uneven crossing over in the male in which the original attachment

of the X's arose. The lines used probably go back to the yellow attached-X which came from a yellow male (L. V. MORGAN 1938). However that may be, the Y chromosome suppresses a very high degree of *spa* for the XXY females in the same experiments were all smooth with the exception of a few rated as 1 in exp. 371 in which the ci^D controls were slightly granular.

The decided effect of the heterochromatic Y chromosome shown by the differences in the manifestation of *spa* in XO and XY, and in XX and XXO as compared with XXY and XXY flies, led to experiments to show the grade of *spa* when only part of a Y chromosome is present.

The long arm of Y contains a mutant allele of *bb* and the short arm contains a normal allele. The loss of a region including the normal allele of *bb* may be detected when a deficient Y is used in combination with an X chromosome which carries *bb*.

A Y known as Y bobbed deficiency (Y^{bb-}) and described as deficient for *bb* lacks two-thirds of the short arm of Y; the wild-type allele of *bb* is included in the deficient region as shown by XXY^{bb-} females that carry a mutant *bb* gene in each X and in the long arm of Y and are visibly *bb*. Such females when homozygous also for *spa* showed if any a very slight degree of *spa* at room temperature. At 19°C *spa* was more decided and was higher in the XY^{bb-} males than in the XXY^{bb-} females.

Another loss of heterochromatin is recognized in so-called "Y sterile" (Y^{st}) which is a rod shaped chromosome cytologically resembling the long arm of Y in length and in having a similar constriction. Y^{st} carries a bobbed allele, X^{bb}; Y^{st} male is *bb* and sterile. Y^{st} is carried in stock of $w^e\ bb^l/w^e bb^l$; Y^{st} females and $w^e bb^l$; Y males. Bobbed females that survive are homozygous for bb^l and carry Y^{st} and are saved from the lethal effect of bobbed lethal (bb^l) by the allele of *bb* which is present in Y^{st}. After ci^D had been introduced into the stock, females were mated to *sc cv v f* bb^l; *spa* males and their *spa*/ci^D offspring were inbred. A few homozygous sparkling females of the F_2 and F_3 generations were obtained. They carried bb^l/bb^l; Y^{st} and were low grade *spa* at 25° and of higher grade at 19°. The heterochromatin of the long arm only of Y did not suppress *spa* when present with two X's carrying bb^l.

Males showing the character *bb* are found (rarely) in *bb* stocks. The manifestation of *bb* may be explained (as in the case of XY^{bb-} and XY^{st} where there is cytological evidence) as due to a deficiency, in the short arm of Y, of a region including the wild-type allele of *bb*.

Three such Y chromosomes have been introduced into *sc bb; spa*/ci^D flies. Y^{bb} was found by BRIDGES in 1926. Y^{bb36} and Y^{bbl} have been more recently found in two different *bb* stocks each in a single male showing *bb*.

XY^{bbl} males raised at 19° showed a very high degree of *spa* but so also did the XX females which can be used as controls for the effect of the Y (table 3, exps. 90a and t). The mode of distribution of males carrying a normal Y is usually well defined and is two or more grades below the mode for females. At 19° an effect lethal to males was present with Y^{bbl} (ratio of the total was 1:13) and there were but seven males in the sample; these were all of grade 5 though very slightly less *spa* than their sisters which were also within grade 5 except

one of grade 6. At 25° the males (in a 1:1 ratio) were more different from the females but again less different from their sisters than are XY males from their sisters.

XY^{bb1} males were directly compared at different temperatures and at different times with XY^{bb36} and XY^{bb}. The expression of *spa* was about the same in all of the males (a little more decided in the flies carrying Y^{bb36}). They all showed the same characteristic, the males were only slightly less *spa* than the XX females as is to be expected if the Y's (as the expression of *bb* in males indicates) were to some extent deficient.

The effect of reduction of heterochromatic content of the X chromosome was also studied; *spa* was rated in normal flies and in sibs which carried X chromosomes known to be deficient for *bb* and for neighboring heterochromatic regions. Such an X is GERSHENSON's bobbed deficiency ($X^{bb\ DfG}$ or X^{-}) in which the locus of *bb* and heterochromatin to the right and left of *bb* are missing. The deficient chromosome is marked by the mutant yellow; and when used with a chromosome which carries *bb*, all classes are distinguishable.

In exp. 94a (table 3) the expression of *spa* in $X^{-}X$ and $X^{-}Y$ offspring of $X^{-}X$ females was markedly higher than in the XX and XY offspring respectively, and the same relation held as between the two classes of females in exp. 104k and as between males in exp. 104n. Reduction of heterochromatin in X was correlated with more extreme *spa*.

Spa was observed also in flies carrying the deficient X found by DOBZHANSKY. Both DOBZHANSKY's and GERSHENSON's chromosomes are deficient for the locus of *bb* and both are visibly shorter in heterozygotes than the normal X.

DOBZHANSKY's deficient chromosome (X^{DfD}) measured two-thirds the length of the normal X in metaphase plates of larval ganglia of females. SCHULTZ (unpublished) observed in salivary gland preparations that breaks of an inversion found by STURTEVANT and BEADLE were at bands 4D1 and 19F and that the deficiency was for the section 20C–D (BRIDGES' salivary chromosome map, 1935). GERSHENSON's deficient X was approximately three-fourths as long as the normal X in oögonial metaphase plates and SCHULTZ (unpublished) observed in salivary chromosomes a deficiency from before 19F to right of 20C. Accordingly, the sections missing from the two chromosomes are not coextensive, and it appears that their effects on the expression of *spa* are also probably different.

The distribution of grades of *spa* when Df^{D} was present was not noted, but the eyes were directly compared at various times with those of flies deficient for Df^{G} and the latter appeared to be rougher in corresponding classes of flies. More definite data might show whether there may be an effect on *spa* of heterochromatin to the left of the locus of *bb*, as this region is absent in GERSHENSON's deficiency but present in DOBZHANSKY's.

A third kind of X which may also be deficient for the locus of *bb* is known as X bobbed lethal (bb^{l}). The extent of a possible deficiency is not known. The chromosome has the same kind of effect on expression of *spa* in heterozygous females and in males as have the two known deficiencies but to a lesser degree than X^{DfG}. This was seen in direct comparisons of *spa* stocks into which each

TABLE 4

Summary of data relating to homozygous spa, modified genotypes.
(At 18°±, at 22°± marked.)*

NO.	P_1 ♀	F_1	TOTAL	X % ABOVE GRADE Y		GRADE OF MODE	F_1	TOTAL	X % ABOVE GRADE Y		GRADE OF MODE
				X	Y				X	Y	
130A	$X^{Dp}XX$	$X^{Dp}XX$	77	4	3	2	$X^{Dp}XY$	19	0	0	
11a	$X^{Dp}XX$	$X^{Dp}XX$	79	2.6	3	2	—				
104k	$X^{-}X$	$X^{-}X$	85	82	3	4–5	$X^{-}Y$	85	68	1	2
104n	$X^{-}X$	—					$X^{-}Y$	23	44	1	1–2
94a	$X^{-}X$	$X^{-}X$	83	100	3	5	$X^{-}Y$	104	53	1	1–2
119k*	XX	$X^{-}X$	28	78	3	4					
119m	$X^{-}X^{-}Y$	$X^{-}X^{-}Y$	92	0	2	1–2	$X^{-}Y$	97	64	2	3
							$X^{-}YY$	64	0	0	
87B	$X^{-}X^{-}Y$	$X^{-}X^{-}Y$	47	0	1	1	$X^{-}Y$	103	100	2	3–4
							$X^{-}YY$	66	0	0	
53B	$X^{-}X^{-}Y$	—					$X^{-}Y$	44	55	2	2–3
							$X^{-}YY$	42	0	0	
53A	$\underline{XX}Y$	$\underline{XX}Y$	n	0	0		$X^{-}Y$	60	100	2	3–4
		$\underline{XX}O$	29	100	4	5–6					
105p	$\underline{XX}Y$	$\underline{XX}Y$	55	0	0		$X^{-}Y$	43	95	2	4
		$\underline{XX}O$	18	89	4	5	$X^{-}YY$	5	0	0	
37l*	$\underline{XX}Y$	$\underline{XX}Y$	40	0	1		$X^{-}Y$	91	72	2	3
		$\underline{XX}O$	21	28	4	4	$X^{-}YY$	16	0	0	
37p	$\underline{XX}Y$	$\underline{XX}Y$	62	0	0		$X^{-}Y$	74	95	2	4
		$\underline{XX}O$	14	79	4	5	$X^{-}YY$	3	0	0	
90a	XX						XY^{bb1}	7	100	4	5
90t*	XX						XY^{bb1}	31	100	1	2–3
114	$\underline{XX}O$	$\underline{XX}Y$	123	0	0		XO	99	100	4	5–6
109	$2^{-}/2$	$2^{-}/2$	47	100	4	5	$2^{-}/2$	46	98	3	4
131a	$2^{-}/2$	$2^{-}/2$	162	70	3	3, 6	$2^{-}/2$	149	84	3	1, 6
131l	2/2	$2^{-}/2$	80	60	3	4, 6	$2^{-}/2$	59	70	3	2, 5
114w	4/o	4/o	15	100	4	5	4/o	70	20	3	2–3
114v	4/4	4/o	27	100	4	5	4/o	92	28	3	3
129a	4/o	4/o	6	100	5	6	4/o	6	100	3	5
129l	4/4	4/o	17	100	4	6	4/o	50	92	3	4

of the three deficiencies in question had been separately introduced.

An effect of heterochromatic deficiency in two X's is visible in $X^{-}X^{-}Y$ females with either deficiency G or D. $\underline{XX}Y$ females and the XXY females that have been examined have smooth eyes, but in the eyes of $X^{DfG}X^{DfG}Y$ females the range of *spa* is from grade 0 to 2 (table 1). They overlap normal, but they show that with the loss of heterochromatin in the two X's the Y does not entirely suppress the character *spa*. $X^{-}XY$ females, however, carrying either of the deficiencies in one of the X's are smooth at room temperature.

The effect of an extra Y appears in half of the males of the $X^{DfG}X^{DfG}Y$ line. The males fall into two large classes (table 1, exps. 119m, 53B, and 87B), one which comprises $X^{-}Y$ males, all above grade 2, and a second class of smooth eyed males which have received a Y from each parent and are $X^{-}YY$ males.

TABLE 5

Summary of data relating to homozygous spa, normal genotypes.
*(At 18° ±, at 22° ± marked *, at 25° marked †.)*

NO.	P_1 ♀	TOTAL NUMBER OF F_1 XX	% ABOVE GRADE 3	GRADE OF MODE	TOTAL NUMBER OF F_1 XY	% ABOVE GRADE 1	GRADE OF MODE
114w	4/o	52	o	2	43	o	
114v	4/4	39	10	2	39	o	
131a	$2^{-}/2$	175	37	1, 4	148	59	1, 5
131l	2/2	90	23	2, 4	57	21	
58a	(74)	140	1.4	3	102	5	
119k*	XX	—			n	o	
104k	X-X	89	25	3	—		
104n	X-X	—			79	o	
94a	X-X	119	58	3–4	78	o	
94k	XX	61	87	4	58	2	
103a	(ext)	81	35	3	88	17	1
130A	$X^{Dp}XX$	90	38	3–4	40, n	7.5	
11a	$X^{Dp}XX$	77	23	3	—		
11m	XX	152	30	3	—		
109	$2^{-}/2$	36	25	3	25	20	1
125a	$4^{-}/4$	40	55	4	40	37	1
128a	$4^{-}/4$	123	81	4	44	67	2
128l	4/4	109	68	5	120	57	2
129a	4/o	47	43	3–4	38	61	2
129l	4/4	113	35	3	44	11	1
57l	$\underline{XX}Y$	—			143	52	1–2
57v	$\underline{XX}Y$	—			158	61	1–2
121a	ci^{D}	66	79	4	50	72	2
121l	not-ci^{D}	195	100	5	161	90	3
120a	ey^{D}	38	92	4	55	53	1–2
120l	not-ey^{D}	159	94	5	121	65	2
59a	(a)	91	90	4	127	79	2
58l	(a)	23	100	5	35	91	3
58s*	(a)	10	o	3	10	o	
23a	(a)	178	76	4	178	98	2–3
23t†	(a)	179	17	2	192	0.2	
58bi*	(75)	42		3, 6	46		0, 5
58y*	(75^{h})	20	100	6	20	100	5–6
58z*	(75^{l})	20	50	2–5	19	32	2
90a	XX	76	100	5	—		
90t*	XX	26	89	4–5	—		

This again is an example of the suppression of the character *spa* in the presence of an extra Y.

The same kind of effect is found also when a duplication for heterochromatin of the X chromosome is present in the nucleus. Dp. (1)101 was chosen as suitable for the experiment because both males and females carrying the duplicating fragment are viable and the fragment consists almost entirely of heterochro-

matin; the euchromatic portion of the chromosome from 2A2 to between 19F4–5 and 20A is missing. The females that carried the duplication (tables 3, 4, 5, exps. 130A, 11a, 11m) showed a lower degree of *spa* than did the controls in the same populations. The modes of distribution were at grade 2 in duplication females and at 3–4 in normal females (exp. 130A). The actual difference due to the duplicating fragment may be greater than the apparent difference because a slight roughness of the eye sometimes occurs in the presence of a duplicating fragment.

If the combinations of sex chromosomes of flies which were rated for *spa* are arranged in sequence according to the modes of frequency of distribution at 18° ± the order is: XO, $\underline{XX}$O, XY^{bb1}, X–X, [X–Y(F_1 of $\underline{XX}$Y)], [X–Y(F_1 of X–X–Y)], XX, $X^{Dp}XX$, X–Y(F_1 of X–X), [XY(F_1 of $\underline{XX}$Y)], XY, X–X–Y, (X–XY, XXY at 22° ±), $\underline{XX}$Y, X–YY(F_1 of $\underline{XX}$Y), X–YY(F_1 of X–X–Y), $X^{Dp}XY$. The combinations in square brackets are those for which there has been no direct control.

Other combinations that have been observed but have not been rated for *spa*, namely, flies carrying Y^{bb}, Y^{bb36}, Y^{bb-}, or X^{DfD}, X^{bb1}, have been found by comparison with measured combinations to fit according to their degrees of expression of *spa*, in an orderly way into the series.

Within the series are subseries the terms of which differ only by the addition of one chromosome element. When the terms in the subseries are arranged in the order of progressive suppression, they form the same sequence as that based on the modes of distribution. The symbol ">" means "is more *spa* than"; the types cited have all been measured by the scale of grades except $\underline{XX}Y^{bb-}$ and XY^{bb-}; these can be used because from direct comparisons it is known that $XO > XY^{bb-} > \underline{XX}Y^{bb-}$, and $\underline{XX}Y^{bb-}$ is slightly *spa* and therefore ranks between $\underline{XX}$O (of very high grade) and $\underline{XX}$Y which is always smooth.

The subseries may be arranged in four groups that show: 1) The effect of addition of a part or whole of a Y to an X or to a male or female complement of chromosomes; $XO > XY^{bb-}$, $XO > XY^{bb1} > XY$, X–Y > X–YY, and $\underline{XX}O > \underline{XX}Y^{bb-} > \underline{XX}Y$; 2) The effect of the addition of an X to an X or to a male complement; XO > XX, X–Y > X–XY, $XY^{bb-} > \underline{XX}Y^{bb-}$, and XY > $\underline{XX}$Y; 3) The effect of the addition of a fragment of X composed for the most part of heterochromatin; XX > $X^{Dp}XX$, XY > $X^{Dp}XY$; 4) The effect of the addition of an X deficient for a large part of the heterochromatin; XO > X–X, X–Y > X–X–Y, and XY > X–XY.

Several conclusions can be drawn; Y suppresses *spa;* a deficient Y has the same kind of effect but to a lesser degree. From 3) it can be concluded that a heterochromatic fragment of X has the same kind of effect as has the heterochromatin of Y.

From 2) it appears that addition of a whole X also has the same kind of effect; this might be expected if it is the ratio of heterochromatin to euchromatin and not the actual amount of heterochromatin which is effective. The ratio of heterochromatin to euchromatin in a whole X is higher than the ratio in the whole normal male or female complement of chromosomes so that the addition of an X, although it contains actually more euchromatin than heterochroma-

tin, increases the ratio of total heterochromatin to total euchromatin. The subseries 4 shows that even the deficient X^- which retains some proximal heterochromatin but is composed almost entirely of euchromatin, likewise acts in the same direction as Y, that is, as a suppressor.

The difference between X and X^{Dp} is a difference in the amount of euchromatin. X^{Dp} contains all of the heterochromatin of X and very little of the euchromatin; therefore if the proportion of heterochromatin to euchromatin is the factor involved in the additions of heterochromatin, the addition of X to the nucleus should be less suppressive than the addition of X^{Dp} and the addition of X^- (largely euchromatic) should have still less effect than has X. The members of the series, X^-XY, XXY, and $X^{Dp}XY$, in which the three elements are present in addition to the combination XY are beyond the threshold of wild type and all three cannot be compared. However, the effects of X and X^- have been measured and with increase in the proportion of euchromatin to heterochromatin in the X, there is less effect of suppression; X^- suppresses *spa* less than does X (cf. X^-Y and XY, X^-X and XX, Table 3, Exps. 94a and 94k).

In no genotype used with *spa* has the ratio of euchromatin to heterochromatin been high enough to enhance *spa*. SCHULTZ (1936) reporting results with variegations mentions "duplications for active regions, some of which annul, others of which stimulate, the effects of the Y chromosome."

The effect of increasing amounts of heterochromatin in the nucleus has been shown to be progressive regardless of sex.

There are besides the subseries showing the effect of addition of one chromosome element, the contrasting pairs of combinations which show a fairly constant relation between females and males, that is between chromosome complements differing in the presence of an X or a Y; XX>XY at 18°± and at 22°±. $X^-X>X^-Y$, $X^{Dp}XX>X^{Dp}XY$, to which may be added in anticipation of the next section, XX Df(2)>XY Df(2) and XX haplo-4>XY haplo-4. If the X or the Y is altered in one sex, the difference between the degrees of *spa* in the two sexes is correspondingly changed, e.g., XX and XY^{bbl} are more alike than are XX and XY. Other changes in the sex chromosome complements are, as noted above, accompanied by corresponding changes in the relative degrees of *spa* in females and males, sometimes shifting the direction of the difference; XY>$\underline{XX}$Y and $XY^{bb-}>\underline{XX}Y^{bb-}$ are examples in which *spa* is more extreme in males than in females.

There is a deficiency for heterochromatin in one of the large autosomes which was tested for an effect upon the expression of *spa*. Df(2)Ms10 which lacks a block of heterochromatin in the proximal region of 2R produces a Minute effect and is lethal when homozygous. When present in flies which were homozygous *spa* (exp. 109), all of the Minute females and 74 percent of the Minute males were of grades 5 and 6 although the distribution indices of the not-deficient controls were average. Two other experiments, 131a and 131l, (tables 3, 4, and 5) were complicated by what appears to have been a mixture of two separate lines. This gave a bimodal effect to the scattered distributions of both classes (Minute and not-Minute) of females and males (apparently

semi-lethal in the higher grade not-*M* males). The combined effect of the two lines and the deficiency for 2R resulted in exceptionally high grades while the distribution index of control females was noticeably low. It is evident (especially from exp. 109 in which there is no complicating factor) that in the presence of the normal amount of heterochromatin of 2R in the control flies the expression of *spa* was decidedly decreased as compared with expression in the deficient flies. This is in agreement with effects of the tested heterochromatic regions of the sex chromosomes.

Df(4)M-4 in salivary chromosomes shows a loss of about ten bands. The locus of *spa* is not included in the missing region, for the eyes of Minute flies heterozygous for *spa* are smooth (table 3) showing the presence of the normal allele in the deficient (Df 4) chromosome. The effect on *spa* of the deficiency cannot be studied unless *spa* can be introduced into the deficient chromosome.

Haplō-4 flies carrying only one dose of *spa* show the character to a high degree; the mode is at 5 or 6 in females and 3 to 4 in males (tables 3 and 4) while at the same time the indices of diplo-4 controls (table 5) are above average. The absence of one fourth chromosome from the genotype is largely a loss of heterochromatin and the enhancing effect might be regarded as due to this loss were it not that the absence of chromosome 4 involves the loss of one dose of *spa* in the diploid fly which introduces another situation. The high degree of *spa* in Dp(2; 4)b described earlier is another example of exaggeration effect. In the latter case the balance between the heterochromatin and euchromatin in the fragment 2L-4 (tip of 2L and the proximal heterochromatic region of 4) might be a factor producing a discernible effect. The effects of different doses of *spa* have not been studied.

It has been stated that the eyes of *spa* do not show a mosaic pattern. A suggestion of what may be mosaicism was sometimes observed in the lowest grades, making classification difficult. When the eye is turned to receive illumination from different directions, a slight roughness sometimes may be seen in an otherwise smooth eye, usually at the edge. When the posterior rim is involved the pattern resembles that of *Cat.* In one male the left eye was of this kind; the rough edge was like the right eye (grade 2). The surface of eyes of some males of derived high lines appeared uneven.

There is a class of mosaics which may be explained, by somatic elimination of a chromosome, as composed of two different genotypes each including the region of an eye. Three males had each one eye of grade 6 and the other eye of a lower grade, one each of 0, 1, 3; these may be explained by elimination of Y from a cell which developed into XO tissue including the grade 6 eye. Two other males had each one eye of grade 0 and the other of grade 5, which is the mode of haplo-4, and the eye of high grade may have been included in haplo-4 tissue resulting from somatic elimination of chromosome 4. The grades are not defined enough to exclude either interpretation of any one of the five mosaics. Two mosaics (incompletely described) had each one eye of grade 4 and the other of grade 6 (the grade of haplo-4 females, while the grade of XO males is 5–6). One mosaic had shorter wing and abdomen on the side of the extreme *spa* eye and the abdomen was extreme *bb*. The fly may have been a gynan-

dromorph by elimination of an X. The abdomen of the other mosaic was shorter on the side of extreme *spa* but the bristles were uniform and the fly was perhaps a female mosaic for haplo-4.

MATERNAL EFFECT

It is known that in some variegations there is a maternal effect of a Y chromosome on the expression of variegation in flies that developed from X eggs of an XXY female. The effect is in the same direction as that of an extra Y in the individual which carries the Y. That is, if a maternal effect is present the expression of variegation is less extreme in flies developed from X eggs of an XXY female than in the progeny of XX sisters of those females.

Experiments were not specifically planned to study the effect of different proportions of heterochromatin in the variants used as P_1 females, but in making experiments for observing the direct effect of altered chromosomes variant and normal females were sometimes used separately as P_1. No indication of a maternal effect was found in these experiments which were not refined enough to detect small effects. The results are given in tables 2, 5 where the contrasted matings are numbered alike but have different letters.

The character ey^D is associated with a heavily banded (euchromatic) homozygous duplication (exps. 120a, 120l). The expression of *spa* was the same in the female progeny of ey^D and not-ey^D females. It was slightly more extreme in males whose mothers had been not-ey^D, an effect opposite to the maternal effect found in variegations.

Other modifications tested for maternal effect were Df(2)Ms10, Df(4)M-4, haplo-4, X^{Dp}; with none of these was there any difference in grades in F_1 when the mothers were different (tables 3, 4, 5).

Homozygous *bb; spa* XX females which had developed from X eggs of XXY and daughters of XX females have been compared but not measured. There was a slight difference but the slightly more extreme *spa* appeared in the daughters of XXY, an effect opposite to the effect seen in variegations due to the presence of Y in the mother.

The XX and XY progeny of X–X and XX females from the same mass culture may be compared in exps. 94a and 94k, table 3. The XX daughters of XX mothers (exp. 94k) showed significantly more extreme *spa* than did the XX daughters of X–X mothers (exp. 94a). Again the difference is opposite to that to be expected if lack of heterochromatin in the mother enhances the expression of *spa* in her XX progeny. The sons showed no difference.

An unexplained difference appeared between X–Y sons of X–X females and X–Y sons of X–X–Y or of XXY females, the last two from matings for which there is no control. X–X females rate about 90 percent above grade 3 (table 4), while a higher proportion of heterochromatin is indicated both in the X–X–Y flies (which overlap normal and are never above grade 2, table 1) and in XXY females which are normal (except a few doubtful cases of grade 1). The X–Y sons of X–X are about 55 percent above grade 1, but the X–Y sons of X–X–Y are about 73 percent grade 2, and X–Y sons of XXY nearly 100 percent above grade 2 (72 percent in one experiment at about 22°). There was a marked

difference in expression of *spa* in the sons of X–X as compared with the sons of the other two kinds of females. The differences among the three sets of males indicated a series in which the expression of *spa* varied not inversely but directly with the proportion of heterochromatin in the mother. But since the three lines of flies were not related, it must be borne in mind that uncontrolled modifiers may have been responsible for the more extreme expression of *spa* found in the progeny of the two kinds of mothers which were most heterochromatic.

DISCUSSION

The genetic study of *spa* has shown that it manifests two characteristics of variegations, one often accompanying mutation, the other so far found to be peculiar to the type of position effect called variegation; *spa* phenotype is variable, and it is affected by the proportion of heterochromatin to euchromatin in the nucleus. Because of these resemblances the properties of variegations are of special interest.

A gene mutation may occur in a rearrangement of chromosomes whether the new chromosomal material which comes to be adjacent to the mutating gene is euchromatic or heterochromatic. In either case the mutation is specific for the locus or region of the gene next to the new contact. But there are differences in the phenotypic effect depending upon whether the material in the new environment within the chromosome is heterochromatic or euchromatic (DEMEREC 1941).

When the new contact is euchromatic only the immediately adjacent locus is affected and the mutant gene is stable. But when the new material adjacent to the mutating gene is heterochromatic, there is a variable phenotypic effect resulting in mosaics of what appear, in white variegations, to be alleles of the mutating gene. Loci beyond the one immediately adjacent to the heterochromatin may also be affected. This may be seen in white variegations because there are other loci in the region which may be marked. The mosaicism is exhibited in somatic tissue as in *Pm, rst*,[3] the white series and many other variegations. MULLER (1930) "found that at least two distinct genetic strains (of white mottled) could immediately be obtained from one original mottled line—a lighter and a darker."

Factors outside of the rearranged chromosome which affect the extent of mosaicism are factors of the environment both of the mutated gene and of the adjacent heterochromatin. Such a factor is the proportion of heterochromatin to euchromatin in the whole chromosome complement.

The maternal effect observed in variegations in comparing XX progeny of XXY females with those of XX females is associated with a difference in the cytoplasmic environments of the maturing nuclei depending on the presence or absence of a Y chromosome.

Lastly variegations are affected by temperature, a developmental factor in the physical environment.

It is not yet known to which, if any, category of known mutations *spa* may

belong. It is inherited in the manner of a recessive gene, point mutation or recessive accompanying rearrangement.

The expression of the gene *spa* is in some sense variable and in this it resembles variegations. It does not show detectable mosaics as do, for example, variegations of the white series and *rst*^{3}, but as with them there is variation among sibs, which with *spa* and with the 4th chromosome character *ci* (STERN 1943) is measurable.

If *spa* eyes are in fact mosaics in which the pattern cannot be distinguished, it would be expected that the two eyes of a pair would often show over-all different grades of expression; but there is high correlation between the two eyes as is true also of the wings of *ci* flies. Two eyes of an individual have not been observed that differ by as much as one grade of the scale chosen for measurement. Both eyes were not always examined, but very large numbers have been compared. On the other hand differences in extent of mosaicism are found, among variegated individuals, and it may be that the differences between individual *spa* flies correspond to those differences.

The proportion of heterochromatin to euchromatin in the chromosomal environment is negatively correlated with the extent of the expression of *spa* which is in agreement with a similar effect in variegations. One example from the graded series showing progressive suppression of *spa* with greater proportions of the heterochromatin of the sex-chromosomes is the difference in expression in XX and XY. It is not a difference between females and males as such, because an XO male is more *spa* than an XX female, XX is more *spa* than an XY male, but the XY male is more *spa* than an XXY female. The expression of *rst*^{3} is also different in males and females, but with *rst*^{3} the mutant character is more extreme in XY males than in XX females.

The temperature at which development takes place affects the expression of *spa* and of *rst*^{3}; both are more extreme at lower temperatures. The relative degrees of expression in XX and XY are maintained at the temperatures observed. The difference is also maintained with *spa* when there is a shift in distribution connected with loss of heterochromatin of the sex-chromosomes or of 2R and in haplo-4.

Another character, cubitus interruptus (*ci*), in chromosome 4 (STERN, 1943) is like *spa* in being variable and the degrees of expression of *ci* and of *spa* are shifted in the same direction in response to temperature. The two mutants differ however in that *ci* is more extreme in males than in females.

Experiments which might have indicated a maternal effect gave inconclusive results.

In summary it may be said that factors responsible for the variability observed in *spa* have not been determined, but that the variability resembles in some sort that seen in variegations. In both instances the variability is affected by the proportion of heterochromatin to euchromatin in the nucleus and also by the temperature at which development takes place, but a maternal effect depending on an effect of Y upon cell cytoplasm of the developing egg has not been observed in *spa*.

The phenotypic expression of the mutant *spa* resembles that of the 4th chromosome mutant *ci* in being variable, enhanced by low temperature during development and also in the constant relative degree of expression between XY males and XX females, though in the reverse direction.

The gene *spa* is in some way related to the dominant *Cat;* the heterozygote shows roughness in the part of the eye which in *Cat* heterozygous for wild type is smooth. LEWIS (1945) has shown that in an eye heterozygous for recessive asteroid (which is also variable) and dominant Star there is an effect of *ast* not seen in *S* when heterozygous for wild type. This phenotypic test of allelism is always inconclusive when applied to the relation between a dominant and a recessive.

The genetic study of *spa* shows the need of further investigation of old lines of *spa* and of new lines that have been derived from them and of salivary gland chromosomes; also there is need of a study of dosage effects and of exceptions that have been obtained from X-rayed flies.

SUMMARY

The phenotype sparkling (*spa*) is associated with a gene in the fourth chromosome of *Drosophila melanogaster*. The gene *spa* is tentatively located by evidence from crosses with T(2; 4)b and from a few salivary preparations in the distal range of region 102C.

Spa is inherited in the manner of a recessive gene, but shows characteristics not common to recessive genes. *Spa* bears some relation to dominant *cat*; the eye of the heterozygote shows a low grade of *spa* anteriorly where the eye of *Cat* is smoother.

Distributions of frequencies of grades of the phenotype *spa* show a single mode. The frequencies in one line which had shown monomodal distribution later showed a bimodal distribution. The line was separated into two which after about 25 generations (with almost no selection) bred true.

Among exceptions from X-rayed males smooth lines and extremely rough lines have been derived.

The degree of expression of *spa* varies in the progeny of pairs of flies. Individual flies differ, but definite mosaicism was not detected in single eyes.

Mosaics in which the two eyes of an individual were different have been seen though rarely. They can be explained by somatic elimination of an X, a Y, or a fourth chromosome.

Spa is more extreme at lower temperatures, 17°–19°, than at 22°–25°. A constant relative difference was found between XY males and XX females at all observed temperatures.

Distribution of males in a controlled temperature experiment indicates that beyond the normal threshold (grade of wild type) there are differences which are not detectable in the phenotype.

The expression of *spa* is less extreme with addition of heterochromatin to the nucleus. Addition of increasing amounts of heterochromatin of the sex chromosomes gives a corresponding graded series of decrease in the degree of

expression of *spa*. The expression is lessened also with increase in the proportion of heterochromatin to euchromatin; X which contains more heterochromatin in proportion to euchromatin than does deficient X^- has the greater effect in suppression of *spa*. The constant difference between XX and XY falls consistently into line with progressive differences correlated with increase in proportion of heterochromatin to euchromatin.

In the absence of normal proximal heterochromatin of 2R *spa* is enhanced.

Spa is of high grade in haplo-4 flies and with Dp(2; 4)b.

Negative results were obtained in experiments which might have indicated a maternal effect.

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THE EIGHTH INTERNATIONAL CONGRESS OF GENETICS will be held in Stockholm in the summer of 1948. The date will be announced later, when more is known about other congresses planned for 1948. Swedish geneticists have formed an Organization Committee for the Congress, with Professor Gunnar Dahlberg, of the University of Uppsala, as chairman, and Professor Gert Bonnier, Institutet för Husdjursförädling, Wiad, Eldtomta, as general secretary.

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